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BIOSORPTION PARAMETERS: CAROB KIBBLES (*CERATONIA SILIQUA*) AS A POTENTIAL BIOSORBENT FOR BORON REMOVAL FROM AQUEOUS SOLUTIONS

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SUPERVISOR

THIS MASTER RESEARCH THESIS WAS CONDUCTED IN THE LABORATORIES OF THE FACULDADE DE CIÊNCIAS E TECHNOLOGIA AT THE UNIVERSIDADE DO ALGARVE UNDER THE SUPERVISION OF PROFESSOR MARIA CLARA COSTA

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I declare to be the author of this work, which is original and unpublished. Authors and works consulted are duly cited in the text and are included in the list of references.

Aknowledgment

To declare sincere gratitud in several words is not enough taking into considerarion all the blessings I received through very special people that were part of this extrordinary experience.

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I am blessed and I deeply thank God, my fortress in whom I trust.

Abstract

This work aimed to investigate the removal of boron by bioadsorption using carob kibbles (*Ceratonia siliqua L.*) biomass, a by-product of a seed flour factory located in Faro, Portugal. Batch experiments were carried out to evaluate the effect of several parameters such as contact time, pH of the solution, initial boron concentration, adsorbant dosage, temperature of the solution and particle size on boron removal. The optimum contact time obtained was 300 min and the bioremoval is favored as the solution pH increases. This removal was found to increase with an increase in the adsorbent dosage. With an initial boron concentration of 100 mg/L, the maximum percentage of removal (67 %) was achieved with the highest adsorbent dosage tested (200 g/L). On the other hand, when using a fixed dosage of biosorbent (50 g/L) the percentage of boron removal decreases as the initial boron concentration was raised from 20 to 200 mg/L. The best removal temperature was 25 ºC and the particle size that binded more boron from the aqueous solution was 0.025 mm.

Particle size distribution showed that the majority of biomass is within the sizes between 0.025 and 0.106 mm.

Keywords: Bioadsorption, Boron, Carob Kibbles.

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List of Abreviations and Symbols

%R	Percentage of recovery
%Rem	Percentage of removal
a	y-intercept
B	Boron
b	Slope
C_{CRM}	Concentration of the Certified Reference Material
$CaCl_2$	Calcium chloride
CCMAR	Center for marine sciences
CCME	Canadian Council of Ministers of the Environment,
Ceq/Ce	Analite concentration in the equilibrium
Co	Sorbate initial concentration
CRM	Certified Reference material
Cs	Sorbent initial concentration
EDS	Energy-dispersive X-ray spectrum
EU	European union
Fcal	Calculated F value for Hartley's Fmax test
Fcrit	Critical F value for Hartley's Fmax test
$FeCl_3$	Iron trichloride
FT-IR	Fourier transform infrared spectroscopy
H_3BO_3	Boric acid
HCl	Hydrochloric acid
He	Helium
HNO_3	Nitric acid
k	Afinity constant
K	Langmuir adsoprtion constant
Ka	Acid dissociation constant of a solution
KBr	Potasium bromide
LOD	Limit of detection
M	Amount of the added (bio)sorbent on the dry basis
MP-AES	Microwave plasma atomic emission spectroscopy
NaCl	Sodium chloride
NaOH	Sodium hydroxide
NMDG	N-methyl-D-glucamine
p^1	Orbital p with one electron
PEUF	Polymer-enhanced hybrid ultrafiltration process
pHf	Final pH
pHi	Initial pH

pKa	The negative base-10 logarithm of the acid dissociation constant (Ka) of a solution
PZC/pH_{pzc}	Point of zero charge
qeq/qe	Adsorption equilibrium capacity
q_{eq}	Bioadsorption capacity
qmax	Maximum adsorption capacity
r	Regression coefficient
rpm	Revolutions per minute
s	Standard deviation
s^2	Orbital s with two electrons
SD	Standard deviation
$SD_{x/y}$	Residual standard deviation
SEM	Scanning electron microscopy
t	Calculated value for student´s t test
t_{crit}	Critical value for student´s t test
UAlg	Universidade do Algarve
V	Volume of the sorbate-bearing solution contacted
WHO	World Health Organization
Xm	The arithmetic average from the analyte measurements
ΔpH	pHinitial – pHfinal

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1. Introduction and Literature Review

1.1 Chemistry of boron in aqueous environments

Boron is the element number five in the periodic table with symbol B, atomic mass of 10.81 and electron configuration [He] $2s^2 2p^1$. It has some of the properties of metals and some of the properties of non-metals, then it is classified as metalloid [1] (Figure 1).

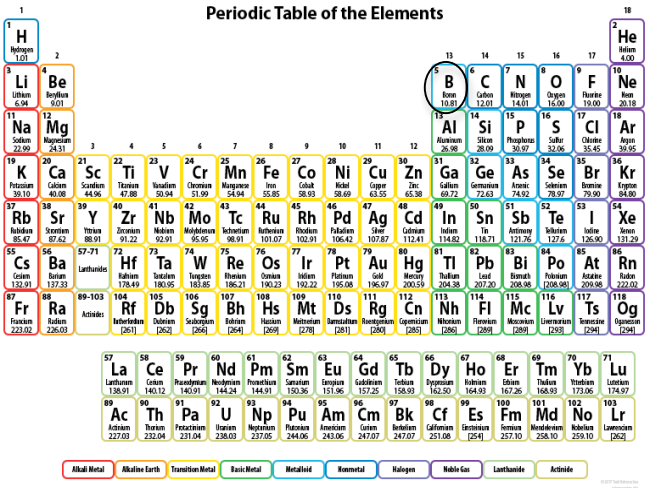
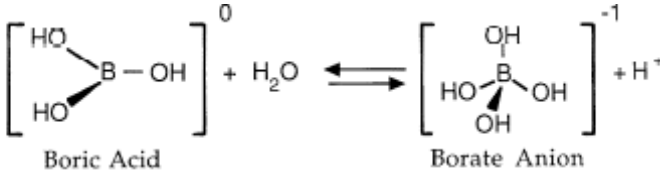


Figure 1. Boron in the periodic table

Boron is not found as free element in nature, for example, in aqueous environments at low concentrations i.e <0.02 mol/L, it is present as boric acid or as borate ions at acidic and alkaline conditions respectively, according to the dissociation reaction as shown in the following equations 1 and 2 [1], [2]:

$$H_3BO_3\,(aq) + H_2O \rightleftharpoons B(OH)_4^-\,(aq) + H(aq)^+ \quad (K_a = 7.3\text{x}10^{-10};\ \text{pKa } 9.1) \qquad (1)$$



Boric Acid Borate Anion (2)

Regarding the origin of the acidity of aqueous boric acid solutions there are some data that lead to several interpretations. Firstly, Raman spectroscopy of strongly alkaline solutions has shown the presence of $B(OH)_4^-$ ions, [3] leading some to conclude that the acidity is exclusively due to the abstraction of OH^- from water [3], [4], [5], [6].

Therefore, boric acid may be characterized as Lewis acidity of boron toward OH^-, rather than as Brønsted acidity [4], [5], [6].

On the other hand, other source [7] says that boric acid is also a tribasic Brønsted acid, with successive ionization steps (equations 3, 4 and 5):

$$B(OH)_3 \rightleftharpoons BO(OH)_2^- + H^+ \ (K_{a1} = 5.8\text{x}10^{-10};\ pK_{a1} = 9.2) \quad (3)$$

$$BO(OH)_2^- \rightleftharpoons BO_2(OH)^{2-} + H^+ \ (K_{a2} = 4\text{x}10^{-13};\ pK_{a2} = 12.4) \quad (4)$$

$$BO_2(OH)^{2-} \rightleftharpoons BO_3^{3-} + H^+ \ (K_{a3} = 4\text{x}10^{-14};\ pK_{a3} = 13.3) \quad (5)$$

Since the value of K_{a1} in equation 3 is comparable to that of the reaction with OH^- in equation 1, the concentrations of $BO(OH)_2^-$ and $B(OH)_4^-$ are similar [7].

Polyborate anions are formed at pH 7–10 if the boron concentration is higher than about 0.025 mol/L [2] (or at 0.4 mol/L according to another reference [8]). The best known of these is the 'tetraborate' ion (equation 6), found in the mineral borax:

$$4[B(OH)_4]^- + 2H^+ \rightleftharpoons [B_4O_5(OH)_4]^{2-} + 7H_2O \quad (6)$$

The fraction diagram (Figure 2) shows the pH dependence of boron species in aqueous solutions [2] (≤0.04 mol/L [8]). It can be noticed that at pH under the pKa value, boric acid predominates and at pH over the pKa borate ions are mainly present in the solution.

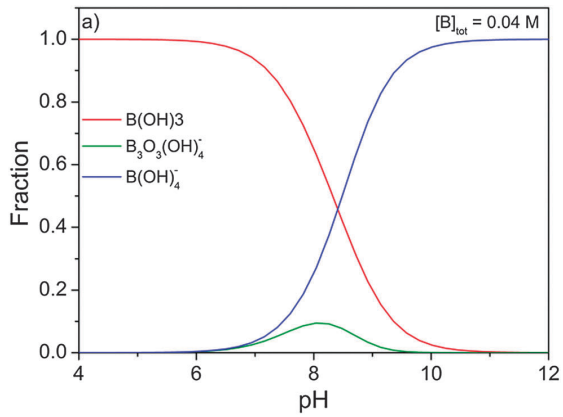


Figure 2. Fraction diagram for aqueous boron species at 0.04 mol/L

According to the fraction diagram (Figure 3) as the pH of the solution changes at significant concentrations (for example, ≥0.4 mol/L), other highly water soluble polyborate ions are found, such as $B(OH)_4^-$, B_3O_3 rings including $B_3O_3(OH)_4^-$, $B_4O_5(OH)_4^{2-}$, and $B_5O_6(OH)_4^-$ [2], [8].

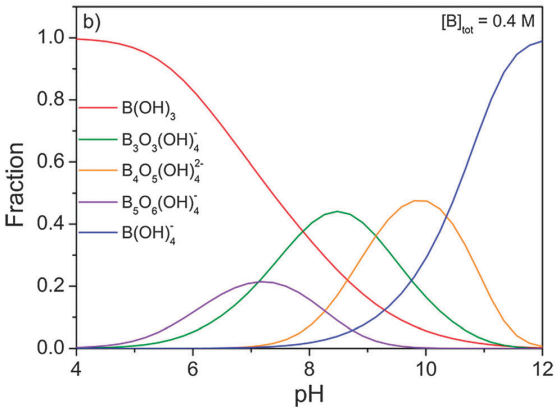


Figure 3. Fraction diagram for aqueous boron species at 0.4 mol/L

1.2 Boron in the environment

Boron is an element widely distributed in the lithosphere, atmosphere and hydrosphere. Nevertheless, has a low abundance on earth. As previously mentioned, it is not found in nature as free element and its natural occurrence is the result of a global biogeochemical cycle as it occurs with other bioelements on earth, though some punctual natural sources can be described for boron presence in lithosphere and hydrosphere (Figure 4) [9].

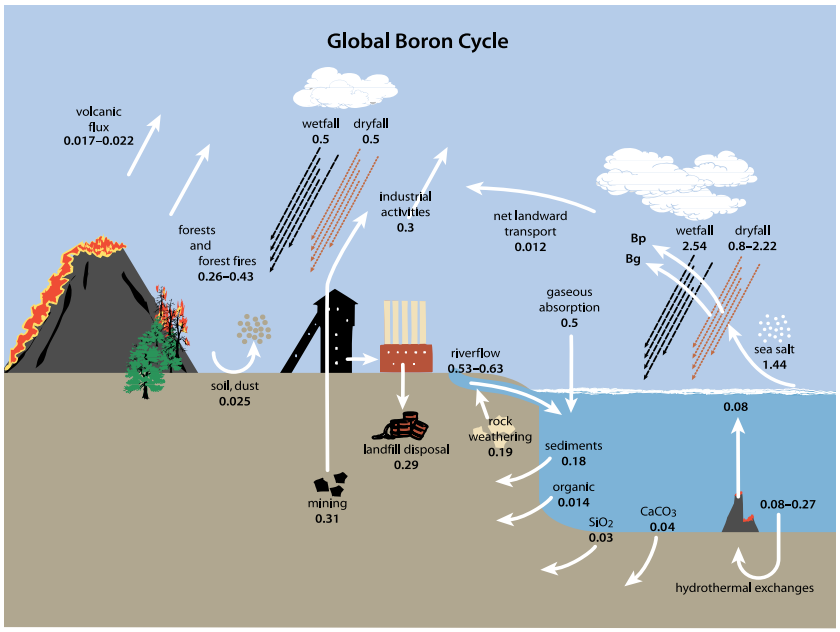


Figure 4. Model for the global biogeochemical cycle of boron (units represent fluxes in Teragrams of boron per year)

Boron concentration in sea water can range from 1-10 mg/L, its concentration in fresh water is usually from less than 0.01 mg/L to 1.5 mg/L and increases significantly in

areas where soil is boron rich. In Europe, the fresh surface water concentration ranges from <0.001 to 2 mg/L with mean values typically below 0.6 mg/L. The boron concentrations in groundwater throughout the world range widely from <0.3 to >100 mg/L depending on the region of the lithosphere. Table 1 presents some average values for boron on environmental matrices as well as some natural sources [9], [10].

Table 1. Average values for boron on environmental matrices and some natural sources [9], [10]

Lithosphere			Average (mg/Kg)
Resulting from	The occurrence of concentrated deposits of borate minerals is intimately connected with past or present volcanic activity and arid climatic conditions are essential for continued preservation of such deposits	Earth´s crust	10
	Most soils have low boron content (<10 mg B/kg), while high boron content soils (10–100 mg B/kg) are usually associated with volcanic activity	Soils	30
Aqueous environment			**Average (mg/L)**
Resulting from	Hydrothermal exchanges, wetfall and dryfall from atmosphere, gaseous absorption	Oceans	4.5
	Leaching from rocks and soils containing borates and borosilicates	Groundwater	<0.3-100>
	Proximity to marine coastal regions, and the geochemical nature of the drainage area, weathering of clay-rich sedimentary rock soils, washing of geological materials or volcanic emissions	Surface water	0.6

Human activities are mobilizing significant quantities of boron on environmental matrices altering its global cycle. In addition, the above mentioned concentrations in surface waters can be significantly increased by anthropogenic sources since the industrial and domestic wastewaters discharged to this natural water bodies can be rich in boron residual products [9].

The list of industries utilising boron compounds is presented in Table 2 [11]. From the list, the two societal uses of boron that account for the most are borosilicate glass and ceramics that up thogether sum 64% [12]. There, boron is used to improve the surface hardness and strength of the final product and during the development of mechanical strength of tiles respectively. Consequently, this are the two principal anthropogenic sources of boron to the environment. A particular wastewater from ceramic industry can have up to 36 mg/L of boron before the treatment process and studies have shown that the ceramic wastewater contains usually between 14 and 15 mg/L of boron [14], [15].

Table 2. Industries utilising boron [11]

Industries	Continue
Borosilicate glass	Fertiliziers
Porcelain	Metallurgic
Wire drawning	Nuclear shielding
Enamels	Cutting fluids and catalysts
Cosmetics	Borohydride fuel cell
Leather	Ultrapure water production
Textile	Photographic chemicals
Paint wood-processing	Carpet production
Detergents and bleaching agent	Weatherproofing wood
Insecticides	Fireproofing fabrics
Preparation of disinfectants	Welding and brazing of metals (Borax)
Preparation of drugs	Boron neutron capture therapy

1.3 Biological activity and toxicity of boron

Since boron is in a constant flux in aquatic and terrestrial ecosystems, living organisms have adapted to some levels of environmental concentrations to guarantee important physiological functions and at trace levels boron has an esential role for the diverse forms of life [12].

Some evidence indicates that as micronutrient for plants, boron plays an important role for the cell wall structure and function, the production of chlorophyll, the carbohydrates metabolism and transport, the synthesis and metabolism of phenolic compounds and is regarded as an essential element for the growth of plants [16]. In animals, boron is required to complete the life cycle of some species, for instance, embryological development in fish and frogs does not proceed normally in the absence of extracellular boron [13]. It also has been found that for higher animals and humans physiological amounts of boron are nutritionally important for the metabolism of certain substances including macrominerals, triglycerides, glucose, nitrogen-containing substances such as amino acids and proteins and reactive oxygen species [13]. Researches have indicated that in humans boron reduces the levels of inflammatory biomarkers [17] and some epidemiological studies suggest that countries with population that consume food rich in boron present lower arthritis incidence [13].

The World Health Organization (WHO) has established an intake of 1-13 mg/day as safe and adequate for a healthy individual [18].

Despite the biological activity, some ecotoxicological studies evidence that exposure to boron concentrations higher than the tolerable by plants and animals, can be toxic in the short and in the long term. The range between boron requirements (deficiency) and toxicity is quite narrow and the tolerances vary among different organisms, i.e. in the case of crop plants, carrot (*Daucus carota L.*) is described as tolerant to boron (4 mg/L in irrigation water) whereas orange (*Citrus sinensis L.*) is considered a sensitive plant to boron (1.0 mg/L) in irrigation water [19]. Relative tolerance of crops to boron is summarized in Table 3 [19].

Table 3. Relative tolerance of crops and ornamentals to boron [19]

Sensitive (1 mg/L of boron in irrigation water)	Semitolerant (2 mg/L of boron in irrigation water)	Tolerant (4 mg/L of boron in irrigation water)
Walnut	Sunflower	Asparagus
Plum	Potato	Palm
Pear	Cotton	Clover
Apple	Tomato	Bean
Grape	Olive	Onion
Cherry	Pea	Cabbage
Peach	Wheat	Lettuce
Apricot	Corn	Carrit
Orange		
Grapefruit		
Lemon		

In this aspect, when boron amount is higher than required its toxicity exerts different effects on the very diverse processes in plants, i.e. alters their metabolism, reduces root cell division, lowers leaf chlorophyll contents and photosynthetic rates and also decreases lignin in vascular plants. In a research conducted with soy beans and sunflower plants, when exposed to boron concentration in excess, 2.5 and 15.0 mg/L respectively, some symptoms of toxicity including lowering growth of the plants, smaller size and unusual color of leaves were observed (Figure 5) [19] [20].

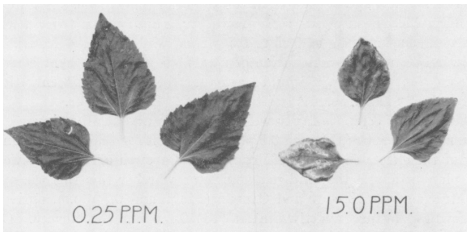


Figure 5. Mature leaves of sunflower plants, showing toxicity of boron at concentration of 15 mg/L

In addition to the adverse effects in plants, during toxicity studies in animals the testes have shown detrimental signs including convulsions, depression and death for acute

exposure to boric acid or borax and studies have demonstrated that the male reproductive tract is a consistent target of toxicity for short-term and long-term oral exposures to boric acid or borax [13]. Other boron toxicological effects in humans as a consequence of the consumption of water and food with increase boron content resulted in creation of problems with cardiovascular, coronary, nervous and reproductive systems [21].

Due to the boron toxicity and potential increasing concentration in surface waters receiving industrial wastewater discharges, regulatory agencies from local and regional goverments have formulated standards for water quality that define the maximun residual levels of boron that should be present in surface, drinking [13], [22], [23], [24] (Table 4), and wastewater. This control has taken an important consideration in order to maintain healthy ecosystems and also for keeping apropriate water supply for human comsumption and irrigation activities. To fulfill those requirements, the industry and the potable water suppliers have focused on technologies and suitable methods to remove as much boron as possible from water and wastewater.

Table 4. Standards for water quality regarding B concentration [13], [22], [23], [24]

WHO	EU Directive		CCME (mg/L)	
Drinking water (mg/L)	Potable water (mg/L)	Water quality for livestock	Freshwater aquatic life Long term	Fresh water aquatic life Short term
2.4	1.0	5.0	1.5	29

WHO: World Health Organisation Guideline
CCME: Canadian Council Ministers of Environment guidelines

1.4 Conventional decontamination methods for boron: advantages and disadvantages

Boron removal technologies for water and wastewater is well documented and some of those technologies take advantage of the chemical properties of boron as a function of pH in order to bind it on an efficient way. According to literature [11] [25], numerous separation technologies have been applied and the methods rely on different operations and conditions including chelating resins [21] (Figure 6), ion exchange resins (Figure 7) [26], adsorption on activated carbon [27], reverse osmosis [26] and the newly developed

hybrid methods [28] (Figure 8), among others. These methods are efficient (up to 99% of B removal). However, in most cases processes development and the maintenance of these operations are expensive or generate considerable volumes of wasted water, which are features not sustainable for the industry.

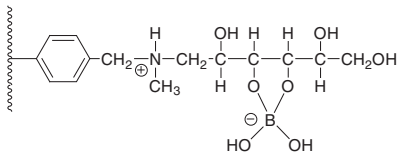


Figure 6. Chelating resins with N-methyl-D-glucamine (NMDG) groups, used to remove boron from aqueous solutions

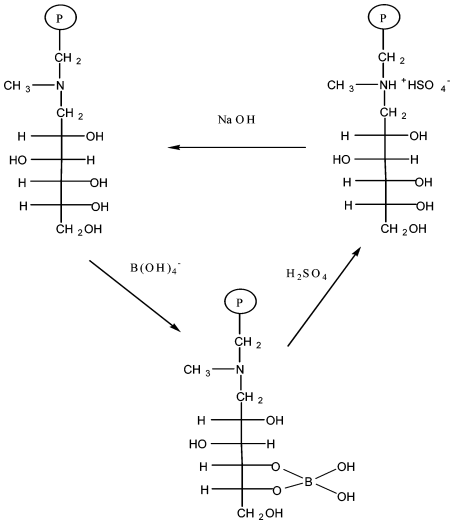


Figure 7. Scheme of the ion exchange of borate with Amberlite IRA 743 resin and regeneration

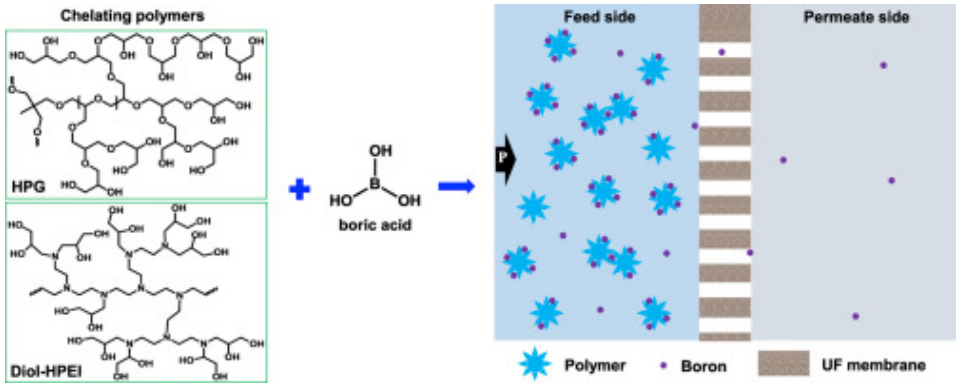


Figure 8. The polymer-enhanced hybrid ultrafiltration (PEUF) process for boron reclamation

Table 5 shows a general view on advantages and disadvantages of technologies for water deboronation [21], [26], [27], [28].

Table 5. Advantages and disadvantages of technologies for water deboronation

Technology	General remark(s)	Advantages	Dissadvantages	Reference
Ion exchange	Borate ion can be removed using an anion exchange resin. I.e; Amberlite IRA-743 (XE-243), has a macroporous polystyrene matrix, on which N-methylglucamine functional groups are attached to boron	Efficient for diluted boron solutions (up to 99 % of boron removal), fast, selective	Expensive, needs further washing of resin for effective reuse/regeneration	26
Adsorption on activated carbon	Better results can be achieved using activated carbon impregnated with chemical substances, bearing in mind certain known properties of boron compounds	Efficient when impregnated with mannitol (up to 99.9 % of boron removal)	Expensive	27
Reverse osmosis	The rejection of boron depends mainly on the pH of the feedwater	Efficient for diluted boron solutions (up to 99 % of total boron rejection)	Expensive, large amount of water wasted by the process	26
Chelating resines	The functional groups of these resins capture boron through a covalent attachment and form a coordination complex	Efficient (93-98 % boron removal)	Expensive	21
Hybrid methods	In these methods, the removal takes place by two stages: sorption of boron on fine bodies plus membrane separation of B loaded bodies	High efficiency (up to 91 % of removal), fast kinetic	Expensive, complex arrange that can affect stability and reliability	28

Taking into account the need of developing effective and low-cost technologies aiming boron removal, the use of other alternatives such as vegetable wastes as sorbent materials can be investigated.

1.5 Biosorption: opportunities and challenges

Biosorption is defined as the adsorption process for species removal (generaly metals or metalloids) or recovery by using biosorbents or biomass, a non living material of biological origin that is used as an adsorbent [29]. This is a surface phenomena that involves a metal or metalloid ion (sorbate) dissolved in a solution (solvent, normally water) that is spontaneously retained by a solid (biosorbent). This process takes place until an equilibrium is established between the amount of solid-bound sorbate species and its portion remaining in solution (at a residual, final or equilibrium concentration) [29]. The degree of the biosorbent 'affinity' for the sorbate determines its distribution between the solid and liquid phases. Figure 9 presents a basic set up for the sorbate uptake when using biomass [adapted from 29].

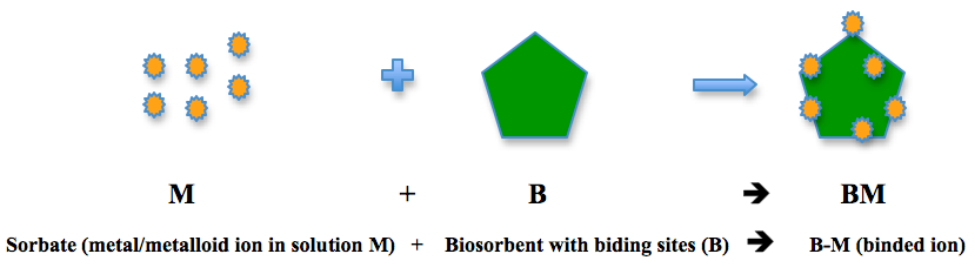


Figure 9. Basic set up for the sorbate uptake when using biomass

There are two widely used calculations to evaluate the sorption performance of biosorption process, percentage of removal of the sorbate from the solution (expressed as % Rem) and adsorption equilibrium capacity of the sorbent (qeq, expresed in mg/g) [29]. The precentage of removal considers the extent of sorbate removed from the solution in the equilibrium at a given time with respect to initial metal/metalloid concentration, whereas the qeq is used to estimate the experimental capacity of the biomass to remove a specific sorbate in the equilibrium. Both calculations are represented in equations 7 and 8 [29].

$$\% \text{ Rem} = \frac{(C_o - C_e)}{C_o} \times 100 \quad (7)$$

Where %Rem is percentage of removal, Co is the initial sorbate concentration (mg/L), Ce is the concentration of sorbate in the equilibrium (mg/L).

$$qeq=(Co-Ceq)*V/m \quad (8)$$

Where qeq is the adsorption equilibrium capacity, Co is the inittial sorbate concentration, Ceq corresponds to the final sorbate concentration (residual) or the sorbate concentration remaining in the solution, V is the volume of the sorbate-bearing solution contacted (batch) with the sorbent (L); and m is the amount of the added (bio)sorbent on the dry basis (g).

1.5.1 Biosorbents

The sources of nonliving biosorbents may include fungi [30], bacteria [31], algae [32], [33] plant biomass [34], [35], [36], [37] and vegetal waste [38], which are usually selected on the basis of availability and low cost and whose biosorption experiment results depict them as natural materials with good biosorption properties over sorbates including metals (Pb(II) [30], [33], Cd (II) [33], [38], Cr(III) [32], Ni(II) [35], Cu(II) [35], [38] and Zn(II) [35]) metalloids (As(III), As(V), [39]), fluoride [40] or dyes [41], etc. In all cases, the sorbates are regularly in ionic state (cationic/anionic), so they can be attached to the sorbent [34]. Sorbates under study are mostly metals or metalloids, since these species pose significant effects on the living organims including humans and still remain challenges for the effective treatment of this ionic pollutants in wastewater.

There is an important number of studies focused on biosorption of metals and metalloids exploring the potential of biomass for reducing the pollution load of aqueous solutions [34]. In all cases, the biosorption is explained due to the affinity between the sorbate ion and the biosorbent whose binding mechanisms depends on the chemical composition of the sorbent surface, the spetiation of the ion, as well as the chemistry of the aqueous solution. Figure 10 illustrates a classification system of the metal biosorption mechanisms [42].

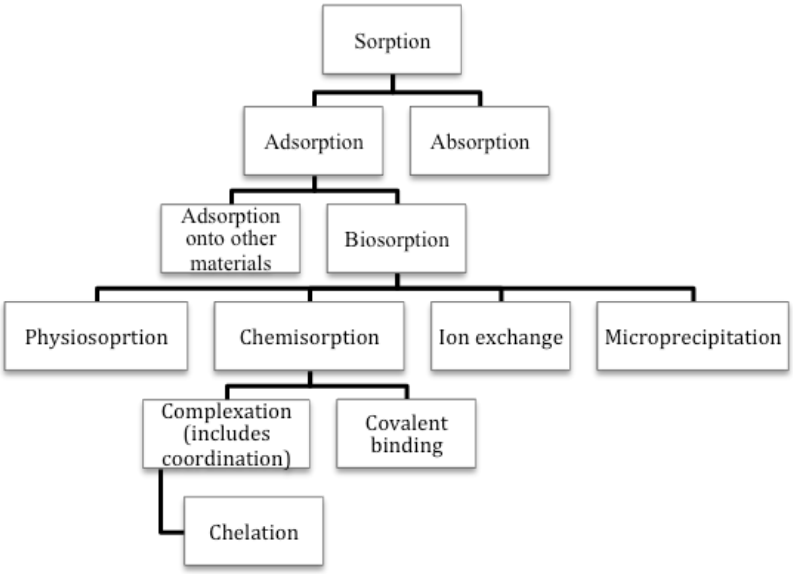


Figure 10. Classification system of metal biosorption mechanisms

1.5.2 Process parameters that affect the biosorption

Although, many variables can influence the effectivness of sorbate biosorption [34], [40] (Figure 11), a few of them have a significant effect. For example, pH is one of the decisive factors that influences not only the chemistry of the solution but also strongly influences the activities of the functional groups on the biosorbent surface, as well as the speciation and the biosorption affinity of metal/metalloid ions. Some of the most important process parameters affecting metal binding are discussed below.

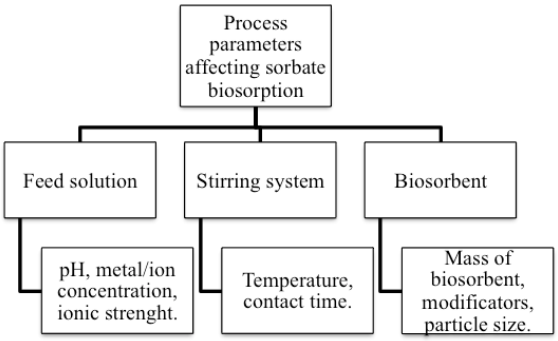


Figure 11. Parameters to assess for experimenting with sorbate uptake by biomass

1.5.2.1 pH

In the experiments, the pH of the sorbate-bearing solution can range from acidity to alkaline conditions. However, attention should be pay to sorbate (i.e; metal/metalloid) behavior as a function of the pH (speciation diagram) in order to prevent undesirable reactions not involving the biosortion process itself, i.e. at pH values over 5.5 because of higher concentration of OH^- ions in the biosorption system chromium (III) ions precipitated as $Cr(OH)_3$. For this reason, in this particular case the experiments should not be conducted at alkaline conditions [32].

The uptake of ions by biomass generally is a function of the solution pH, which influences both cell surface sorbate binding sites and sorbate chemistry in aqueous solutions [32].

When the pH value increases the proton concentration decreases and the biomass surface is more negatively charged. In this context, the biosorption of the positively charged ions increase till reaching a maximum biosorption around [43]. It is accepted that the sorption of metal/metalloid cations (*e.g.* Cd, Cu, Zn) increases with increasing pH and only those ions which can occur as negatively charged complexes or that have a strong "b" character (i.e. tendency to form strong covalent bonds), may show either a decrease in binding with increasing pH or may have no significant pH effect at all [43]. Alternatively, in acidic conditions the biomass surface is more positively charged and the removal of anions increases due to the electrostatic attractions between opposite charges. Table 6 presents some optimal pH values found for the biosorption of some cations and anions including metals, arsenic and non metals (F^- and indosol yellow, dye).

Table 6. Optimal pH values found for the biosorption of some ions including metals and metalloid

Metal/metalloid	Biomass source	Optimal pH	Reference
Pb(II)	Fungi, Candida albicans	5	30
Cr(III)	Macroalgae, Enteromorpha prolifera	5	32
Cd(II) Pb(II)	Algae, Anabaena sphaerica	5.5 3	33
Cu(II) Cr(III)	Vegetal waste, peanut shell	5	38
Cd(II) Ni(II) Cu(II) Zn(II) Pb(II)	Plant, Macrophytes	6	35
Cd(II) Cu(II) Zn(II) Ni(II) Pb(II) Cr(II)	Plant, Macrophytes	6	36
As(III) AS(V)	Agricultural residue 'rice polish'	7 4	39
F^-	Fungi, Pleurotus eryngii	2	40
Indosol Yellow BG dye	Peanut husk	2	41
$HCrO_4^-$	Vegetal waste, Macadamia shell	2	44

1.5.2.2 Sorbate concentration

This parameter also plays a significant role in the biosorption process. It acts as an important driving force to prevail over all mass transfer resistance of all ions between aqueous and solid phases. The higher initial ion concentrations may result in boosting up the biosorption process [43]. Then, sorbate removal increases with the increase in ion concentration [41], [43], but a decrease in sorption of ions at very high concentration can also be observed due to the saturation of active binding sites [44]. Increasing concentration of the solute in the solution could reduce the diffusion of solute in the boundary layer and to enhance the diffusion in the solid. At very high sorbate concentrations, the available sites for biosorption became fewer and the saturation of the sorption sites may be observed [32].

1.5.2.3 Temperature

To investigate the effect of temperature, the biosorption of ions onto biomass is generally studied at different temperatures between 15 °C and 60 °C [32], [39], [40], but experiments at higher temperatures can cause a change in the texture of the sorbent and a loss in the sorption capacity [43]. Temperature affects the biosorption equilibrium allowing a lower or higher retention of the sorbate due to the effect over the frequency of interactions between biosorbent and sorbate [32]. In this sense, the effect can be significant or not, but generally is not strong [32], [40].

1.5.2.4 Contact time

During the biosorption of ions onto biomass the contact time runs from minutes to several hours until it is observed that the concentration of the ions in the solution is relatively constant, this implies that contact time or contact period between the ions in solution and the biomass should be enough for the ions to attain biosorption equilibrium [32], [39], [44] and subsequently to obtain the maximum uptake.

It can also be find that the uptake of adsorbate species is generally rapid in the initial stages and gradually decreases with the lapse of time until equilibrium [39].

1.5.2.5 Mass of biosorbent

The amount of biosorbent solid is important to guarantee that enough adsoption sites are available to adsorb the sorbate concentration desired. Hence, different biomass dosages are applied to study the influence of biomass concentration on the removal of ions by biosorbent [32]. From literature sources, it is expected that the biosorption of ions is increased with subsequent increasing the biosorbent dose until the uptake becomes constant [33], [38], [40], [45].

1.5.2.6 Particle size

The contact surface between any sorbent and the liquid phase plays an important role in the phenomena of adsorption. For instance, the rate of sorbate biosorption usually increases with the reduction in the diameter of the particles, which probably occurs due to the increase of the number of active sites and thus the increase of the biosorbent surface [46]. In this sense, a maximum elimination rate of sorbate may be reached using fine particles [46]

1.5.2.7 Modificators

The biosorbent can be used without previous treatment, but also a modifier to customize the chemical properties of its surface can also be used in order to increase adsorption capacity of the adsorbent. In general, biosorbents are usually prepared from the naturally abundant waste biomass by inactivation and are usually pretreated by washing with acid or base before the final drying [34].

1.6 Biosorption opportunities for boron removal

Essentially, the biosorption process is presented as an alternate and ecofriendly solution, since it can be selective, effective and also economical for the removal of metal/metalloid ions in solution. In this context, the use of biomass for boron removal from aqueous solutions has been studied under different conditions using various biosorbents prepared from Olive bagasse [47], cotton [48], rice husk [49], algae [50] and organic waste materials [51]. During these biosorption studies the effects of process parameters such as pH, from acidic to alkaline conditions, contact time (up to 48 hours), initial boron concentrations, biosorbent concentration, temperature and particle size have been analyzed over boron biosorption. In general, the results are promising and for example a specific biomass (Marine seaweed: Caulerpa racemosa var. Cylindracea) [50] can reach up to 63 % of boron uptake from solution. However, the studies on boron binding by biosorbents still remain a few.

Thus, taking into account the possibilities that the nature can offer for sources of biomass and also considering that boron presents a particular chemical behavior, studies of boron bisorption are extremly pertinent, thus contributing to find new boron low cost process for its removal.

1.7 Carob (*Ceratonia Siliqua L.*): characteristics and potential as biosorbent

The scientific name of carob tree (*Ceratonia siliqua* L.) derives from Greek *keras*, horn, and Latin *siliqua*, alluding to the hardness and shape of the pod. The genus *Ceratonia* belongs to the family Leguminosae (syn. Fabaceae) of the order Rosales (Figure 12) [52].

Figure 12. Carob *(Ceratonia siliqua L)*

The Carob tree is an important component of the Mediterranean vegetation and its cultivation in marginal and prevailing calcareous soils of the Mediterranean region is important environmentally and economically [52]. Its cultivation and production are

centered in Spain, Italy, Portugal, Northern Africa, the larger Mediterranean islands, Iran and the Canary Islands and Macaronesia, where due to its chemical composition it is used by the food and pharmaceutical industry [53]. Figure 13 shows world carob distribution and centers of origin.

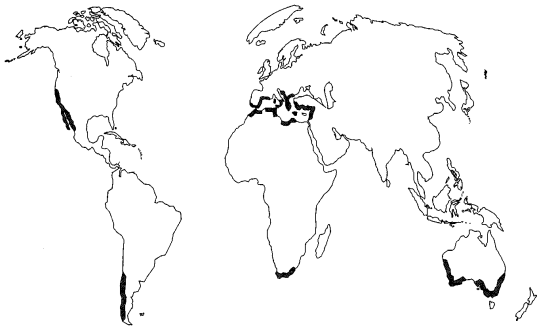


Figure 13. World carob distribution and centers of origin

In producing countries, carob pods have traditionally been used as animal and human food and currently the main use is the seed for gum extraction and this gum is added as thickener, stabilizer or flavoring in food [54]. Carob kibbles (deseeded chopped pods) may be regarded as an agro food waste material rich in sugars (48–56 %), polyphenols (19 ±3 mg/g) and an appreciable amount of protein (mean value 3±2 %) [54], [55].

Some the major polyphenols detected in carob kibbles have been tentatively identified [54] (Figure 14).

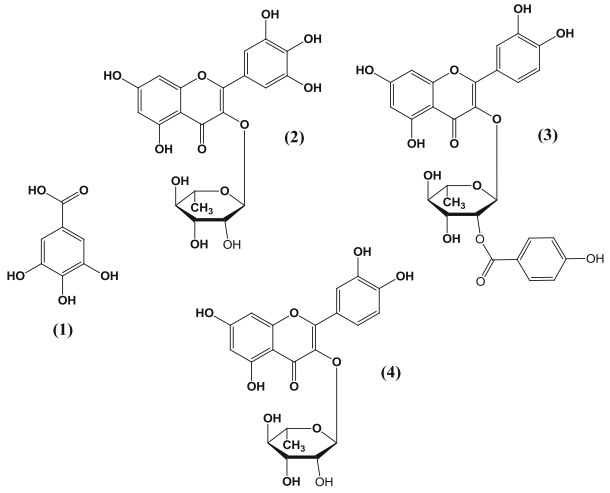


Figure 14. Chemical structures of the polyphenols tentatively identified in carob kibbles, using liquid chromatography–diode array–mass spectroscopy. Assignments: (1), gallic acid; (2), myricetin 3-O-rhamnoside; (3), quercetin p-hydroxy benzoyl 3-O- rhamnoside; (4) quercetin 3-O- rhamnoside

According to the literature, carob pod production worldwide amounts to nearly 400000 tons per year from about 200000 ha. Carob tree plays an important role in the economy of the south of Portugal (Algarve) where 50000 tons of carob fruit is produced each year, making the region the third largest producer in the world [56]. Thus, the waste generated by the industry presents a challenge for its utilization.

Recent research underlines the use of carob for biosorption of some metals such as Ni(II), Zn(II), Cu(II) and Cd(II) [57]. From the results, the maximum biosorption capacity calculated from the Langmuir isotherm was 42.19 mg/g, 31.35 mg/g, 21.65 mg/g and 14.27 mg/g for Ni(II), Zn(II), Cu(II) and Cd(II), respectively at optimum conditions [57]. It was also found that activated locust bean (carob kibbles) with phosphoric acid can remove significant amounts of Cd(II) and Cr (VI). The adsorbent recorded a maximum capacity of 3.17 mg/g (63.3 %) for Cd(II) and 4.13 mg/g (82.5 %) for Cr(VI) at 0.8 g dosage [58].

In a study made with alkaline and acid treated carob shells (5 g/L of biosorbent dose) an important performance for binding cadmium(II) and cobalt(II) was observed. The biosorption yield of Cd(II) was 75.88 %, 20.02 % and 94.39 % for non treated-carob, HCl-tretated carob and NaOH treated-carob, respectively. While for Co(II), the biosorption yield was 37.81 %, 19.50 % and 63.40 %, respectively [59].

On the basis of the above mentioned evidence, it can be underlined that carob kibbles can be explored as an ecofriendly and alternative source of biomass for the removal of pollutants such as boron from water or wastewater. In fact, according to our knowledge, carob kibbles have not previously been investigated for that purpose.

2. Objective

The present work investigates for the first time to our knowledge the effects of contact time, initial pH of solution, initial boron concentration, biosorbent dose, temperature of solution and particle size of biomass, for boron removal from aqueous solutions by using non modified carob kibbles as biosorbent. For the present study, the source of biomass corresponds to carob kibbles from the wastes resulting from a local agro food industry which also allows to valorize this waste as a good and low cost alternative technique to remove boron, an element that is not easy to remove due to its particular chemical behavior, thus for which it is important to develop new and economic atractive removal strategies.

3. Materials and methods

3.1 Biosorbent

Carob kibbles (Ceratonia Siliqua L.) were collected from the waste of a local agro food industry called Industrial Farense Lda, in Faro, Portugal (Figure 15). This factory uses mainly the seeds to extract gum used as a common stabiliser known as E410, which has several industrial applications.

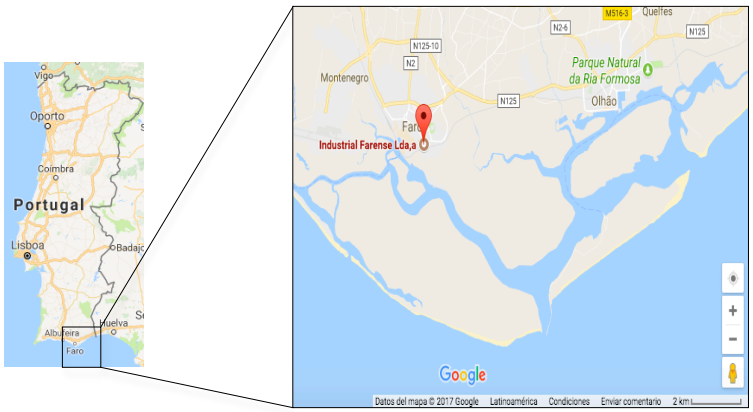


Figure 15. Map with the location of Industrial Farense Lda,a in Faro, Portugal

The obtained material was dried 2-3 h at 50 ºC, deseeded if needed then crashed and sieved with a blender and was directly used as biosorbent without any pre-treatment (Figure 16). A sample was characterized by Fourier Transform infrared spectroscopy (FTIR) and Scanning Electron Microscopy (SEM). The point of zero charge and the particle size distribution were also determined for the biomass.

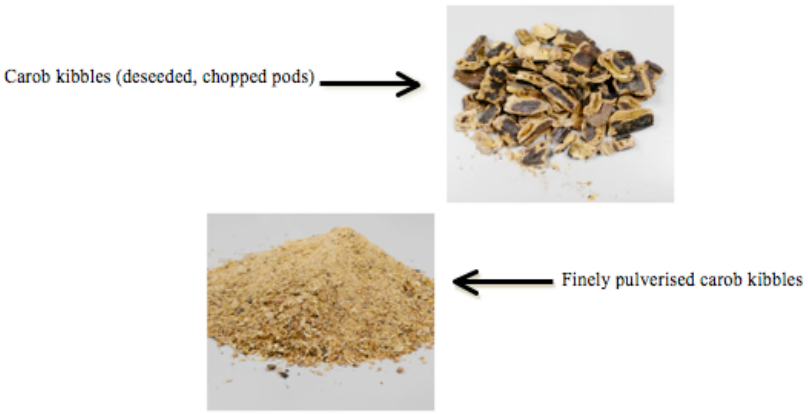


Figure 16. Biomass (pulverised carob kibbles)

3.2 Chemicals

All reagents used in this study were of analytical degree and were used without further purification. Distilled water (Millipore-USA) was used for all the dilutions and for making all the solutions.

All the glassware used in the experiments were cleaned with detergent, thoroughly rinsed with tap water, soaked overnight in 10 % HNO_3 solution and finally rinsed with distilled water.

A stock solution (100 mg/L) of boron was prepared by dissolving Certified Reference Material (CRM) boron standard solution as boric acid (Merck, Germany) in distilled water. The working solutions were obtained by diluting the stock solution with distilled water. Fresh dilutions were prepared and used for each experiment. This CRM was used to build the calibration curves and to prepare the control samples.

Boric acid solution (1000 mg/L of boron) was prepared by dissolving boric acid (Merck, Germany) in the distilled water. Further solutions were freshly prepared for each experimental run. This solution was used as the sorbate for the batch experiments.

3.3 Biomass characterization

3.3.1 Fourier transform infrared (FTIR) spectroscopy

Functional groups of the biosorbent were examined by FTIR using a Bruker Tensor 27 FT-IR-spectrophotometer, in the range between 400 and 4000 cm^{-1} with a resolution of 4 cm^{-1}. An average of 98 scans has been carried out for each sample. The samples were prepared on a KBr pellet [50] in a manual hydraulic press under a pressure of aproximately 10 tons.

The same experiment was carried out with the biomass boron loaded from batch experiments.

3.3.2 Scanning electron microscopy (SEM)

Biomass of carob kibbles enriched with boron and untreated was examined using scanning electron microscopy. Samples of the carob kibbles were placed on an appropriate stub, and thereafter gold-sputtered. The Scanning Electron Microscopy (SEM) couple with Energy-dispersive X-ray spectroscopy (EDS) analysis was carried

out in a FEG-SEM of JEOL JSM7001F instrument, in order to obtain some information about elemental constitution of the samples. The X-ray spectrum of each particle was obtained.

3.3.3 Point of zero charge (PZC)

The point of zero charge (PZC) corresponds to the pH at which the surface of biosorbent exhibits a neutral charge. The PZC of the carob kibbles was determined by the solid addition method [60]. To a solution of KNO_3 (50 mL, 0.1 M) contained in a conical flasks of 100 mL, sodium hydroxide or hydrochloric acid (0.1 M) was added until the initial pH (pHi) of the solution was adjusted in the range from 1.8 to 12.0. The biomass powder was added (0.1 g) and the mixture was left during 48 h with constant shaking at room temperature. The final pH of the solution was registered. The difference between the initial and final pH (pH_f) values ($pH = pH_i - pH_f$) was plotted against the pH_i. The point of intersection of the resulting curve with abscissa at which pH = 0, gave the pH_{pzc}.

3.3.4 Particle size distribution

The particle size distribution was made by granulometry analysis. After dried in an oven and crashed in a blender, the carob kibbles obtained (150 g) were grinded into small particles and sieved (10 min) in a Vibratory Sieve Shaker (Retsch, AS 200) by using screens with mesh sizes between 0.025 and 2.0 mm, 7 sieves of different sizes: 0.025, 0.106, 0.200, 0.250, 0.500, 1.0, 2.0 mm. The mass of carob retained in each screen was weighted and related to the total mass of carob used to perform the essay. The same experiment was carried out for three times and the average value was considered as the final result.

3.4 Adsorption studies

Laboratory batch experiments were carried out to study the biosorption of boron from aqueous solutions. To a solution containing boric acid (100 mL, 100 mg/L) and a specified pH, 5 g of carob kibbles powder was added in a series of 200 mL conical flasks to obtain a biomas dose of 50 g/L. The mixture was kept under a constant speed of 150 rpm for all the experiments in a temperature controlled water bath with shaker

(Julabo, SW 200) during a certain period of time at 25 ºC. All the experiments were carried out in duplicates (otherwise it is specified). The results were considered if a relative standard deviation of the arithmetic average did not exceed 5 %. The presented data are the arithmetic average from two measurements (otherwise it is specified). The batch experiments were performed in the Laboratory of Environmental Technologies of the the Center of Marine Sciences (CCMAR) at the University of Algarve (UAlg), Faro, Portugal.

Before instrumental analysis the pH of the solution was adjusted to values <2.0 with HNO_3 (65 %). Different experimental parameters were studied in order to identify the optimal boron removal conditions.

The contact time was tested at initial boron concentration (Co) of 100 mg/L, at biosorbent dose (Cs) of 50 g/L at 25 ºC and different pH values (3; 5.5; 7.5; 9.5; 11.5). The 0.1 M NaOH and 0.1 M HCl were used to adjust the pH of the boron solution. This also served to investigate the effect of initial pH of the solution on the boron adsorption. pH measurements were performed by potentiometric method, using the pH/Eh Meter (GPL 21, Crison).

Kinetic tests were carried out by contacting 5 g adsorbent with 100 mL of H_3BO_3 solution (100 mg/L of boron) at 25 °C and original pH (5.5) in water bath with shaker (150 rpm). The concentration of boron in the supernatant was determined at different time intervals until optimum contact time was reached.

In order to describe the equilibrium characteristics of boron binding into carob kibbles, adsorption studies were also done to check the effect of initial boron concentration (Co) on boron bioremoval. In this set of experiments, the Co tested were 20, 40, 60, 100 and 200 mg/L at optimum pH, optimum contact time, Cs 50 g/L, at 25 ºC.

The effect of biomass dosage (Cs) over the boron biosorption was done by testing different biomass:solution ratios (1, 5, 10, 15 and 20 g in 100 mL solution) at optimum pH, optimum contact time, at Co 100 mg/L, and 25 ºC.

To determine the effect of temperature, the experiments were performed at three

different temperatures (25, 45 and 55 °C). The set was done at optimum pH, optimum contact time, at Co 100 mg/L and Cs 50 g/L.

The effect of particle size on boron uptake was completed by testing five different particle sizes (>1.0-2.0, >0.5-1.0, >0.2-0.5, >0.106-0.2, >0.025-0.106 mm) at optimum pH, optimum contact time, at Co 100 mg/L, Cs 50 g/L and 25 ºC.

3.5 Boron removal from real samples

The batch experiments aiming to bioremove boron from real wastewater solutions were done using wastewater obtained from ENDEKA ceramics, an industry located in Castellon, Spain; and contacting the solutions with the biomass at optimum pH, optimum contact time, optimal particle size, Cs 200 g/L, 25 ºC and 150 rpm. Two types of wastewater were put in contact with boron: effluent before treatment (with pH equal to 10.45) and effluent after treatment (with pH equal to 7.40). A characterization of the samples, was done in the Laboratory of environmental technologies of the CCMAR in the University of Algarve by staff of the laboratory.

Before the experiments, both real solutions were spiked with 250 mL of boric acid (100 mg/L) and filled to 500 mL according to Table 7. The spiking was done in oder to avoid matrix effect and also to confirm the signal of the analyte (B). In this set of experiments, also a fortified blanck was prepared with the CRM to evaluate the recovery of the procedure. The experiments were carried out in triplicate and the relative standard deviation of the measurement did not exceed 5 %. The presented data are the arithmetic average from three measurements.

Table 7. Spike of real samples for boron removal

	Sample Solution		Spike Solution		Final Solution	
Sample	Volume (mL)	Boron concentration (mg/L)	Volume (mL)	Boron concentration (mg/L)	Volume (mL)	Total final boron concentration (mg/L)
Effluent before water treatment	250	36	250	100	500	68
Effluent after water treatment	250	19	250	100	500	59.5
Fortified blanck	-	-	100	100	-	-

3.6 Batch experiments for boron removal to compare two lots of carob kibbles

Two lots of carob kibbles were compared on the basis of their perfomance to remove boron from aqueous solutions. Both lots were collected from the same industry Industrial Farense Lda, but at different times. One was collected in the beginning of the year 2017 (carob A) and the other was collected by the middle of the year. (carob B).

The batch experiments were done at pH 11.5, Co 100 mg B/L, Cs 50 g/L and at 25 ºC. The experiments were done in sixplicate for each lote for a total of 12 samples. The t-Test: Paired Two Sample for Means, two tailed distribution was applied to compare the results of both lots in order to assess if there is a significant difference between them.

3.7 Instrumental analysis

Boron concentration in the extract was determined by Microwave Plasma Atomic Emission Spectroscopy (MP-AES) using an Agilent 4100 equipment as outlined by the method reported by [61]. The analysis were preformed in the instrumental laboratory of Centre of Marine Sciences (CCMAR) in the University of Algarve (UAlg), Faro, Portugal.

The instrument operating conditions are shown in Table 11.

Table 8. Microwave Plasma Atomic Emission Spectroscopy (MP-AES) operating conditions

Analyte	Wavelenght (nm)	Read time (s)	Nebulizer flow (L/min)	Background correction	Calibration fit
B	249.772	3	0.75	Auto	Linear Through Blanc

To ensure the suitability of the instrument response, calibration curves were prepared from the boron CRM as calibration standard with a minimum of six different concentrations. The highest and lowest concentrations were selected keeping in mind the predicted variation of analyte concentrations The acceptance criterion for calibration curve correlation coefficient (r) was 0.999. For instrument response verification, a calibration standard with known concentration was read as a sample.

The pH was determined by potentiometric method, using the pH/Eh Meter (Crison, GLP 21). The instrument was calibrated and verified according to instrument´s manufacturer recommendation.

Procedures for quality control during the experiments included duplicate (or triplicate or sixplicate) samples, control samples during the batch analysis, instrument calibration and verification, the use of a Certified Reference Material (CRM) and a recovery essay.

3.8 Calculations

To express the amount of boron biosorbed from the solution, calculations are based on % removal. From the instrumental measured concentration of boron, the percentage of removal of boron (% Rem) was calculated using equation 9 [44], [50]:

$$\%Rem=(Co-Ce)*100/Co \quad (9)$$

Where %Rem is the percentage of removal, Co is the inittial boron concentration (mg/L), Ceq corresponds to the final boron concentration (residual) or the boron concentration remaining in the solution (mg/L).

Calculations to determine the limit of detection were based on residual standard deviation (SDy/x) for n=x and on the slope, b, of the calibration curve [62], equation 10:

$$LOD= 3.1\ SDx/y/b \quad (10)$$

The StatPlus was used for the satatistical analysis.

The percentage of recovery (%R) was calculated according to the folowing equation 11 [62].

$$\%R= (Xdet/CCRM) * 100 \quad (11)$$

Where Xde is the arithmetic average from the analyte measurements (mg/L), CCRM is the concentration of the CRM (real value of the analyte) (mg/L).

3.9 Kinetic studies

Biosorption kinetics demonstrates the rate of solutes bonding on the surface of the biological materials [34]. The description of the kinetics of biosorption is complex due to the many facets of the process. Kinetics studies usually provide the important information about the possible mechanism of biosorption that involves the diffusion (bulk, external, and intraparticle) and chemical reactions [34].

Among the various kinetic models that are available in the reported literature, the models based on the order of chemical reaction are of particular interest (Table 8),

especially the Lagergren (pseudo-I-order, PFO) (equation 12) and Ho (pseudo-II-order, PSO) models (equation. 13) [34]. These models are based on the assumption that the rate of sorption is proportional to the number of free sites on the surface of the sorbent in the proper power (first or second) [34].

Table 9. Kinetic models based on the order of the reactions [34]

Model		Differential equation	Nonlinear equation
PFO	(12)	$\frac{dq_t}{dt} = k_1(q_e - q_t)$	$q_t = q_e\left(1 - e^{-k_1 t}\right)$
PSO	(13)	$\frac{dq_t}{dt} = k_2(q_e - q_t)^2$	$q_t = q_e \frac{q_e k_2 t}{1 + q_e k_2 t}$
GO	(14)	$\frac{dq_t}{dt} = k_n(q_e - q_t)^n$	$q_t = q_e - \left[q_e^{1-n} - (1-n)k_n t\right]^{\frac{1}{(1-n)}}$

Kinetics of biosorption of boron by biomass was described by pseudo-second order model (equation 13).

3.10 Adsorption isotherm of linear Langmuir

Sorption isotherms are plots between the sorption uptake (q) and the final equilibrium concentration of the residual boron remaining in the solution (Ceq). In order to describe the behavior of biosorption equilibrium of boron onto carob kibbles the Langmuir equation (equation 15) [29], [32] was applied to the sorption equilibrium results.

$$qmod = (qmax*K*Ceq)/(1+K*Ceq) \quad (15)$$

Where qmod (mg/g) is the adsorption equilibrium capacity for the model, qmax corresponds to the maximun sorbate uptake under the given conditions, Ceq corresponds to the final boron concentration or the sorbate concentration remaining in the solution (mg/L) and K (L/mg) is the Langmuir adsoprtion constant corresponding to the binding capacity .

The qmax and K were calculated from the linearization of Langmuir equation as follows.

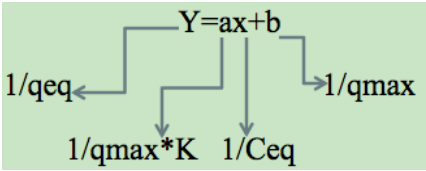
- A plot from 1/qeq vs 1/Ceq was made to obtain a linear regression y=ax+b.

Here, the qeq (mg/g) corresponds to the adsorption equilibrium capacity (experimental) and is calculated from equation 16 [29].

$$qeq=(Co-Ceq)*V/m \quad (16)$$

Where Co is the initial sorbate concentration (mg/L), Ceq corresponds to the final sorbate concentration or the sorbate concentration remaining in the solution (mg/L), both Co and Ceq are analytically determined, V is the volume of the sorbate-bearing solution contacted (batch) with the sorbent (L); and m is the amount of the added (bio)sorbent on the dry basis (g).

- Once the qeq was calculated and the plot 1/qeq vs 1/Ceq is made, qmax and K were calculated on the basis of the linearization of Langmuir equation:



In this sense, equation 17 corresponds to the linearization of Langmuir equation.

$$1/qeq=1/qmax*K\ *Ceq + 1/qmax \quad (17)$$

Where qmax and K are obtained with equations 18 and 19, respectively

$$qmax=1/b \quad (18)$$

$$K=1/qmax*a \quad (19)$$

Finally, to obtain the Lagnmuir isotherm a graph of qmod vs Ceq was plotted.

3.11 Adsorption isotherm of Freundlich

The linearization of Freundlich isotherm is presented in equation 20 [32].

$$lnqeq = lnk+/(1+n*lnCeq) \quad (20)$$

Where, lnqeq corresponds to the natural logarithm of the adsorption equilibrium capacity, lnk is the natural logarithm of the adsorption capacity, n is the adsorption intensity and lnCeq corresponds to the natural logarithm of the final sorbate concentration.

4. Results and discussion

4.1 Characterization of carob kibbles

4.1.1 FT-IR analysis of the biosorbent

The presence of different functional groups in the structure of carob kibbles biomass has been proved by using FT-IR spectra, recorded for dry sample (Figure 17), which allowed observing several important peaks.

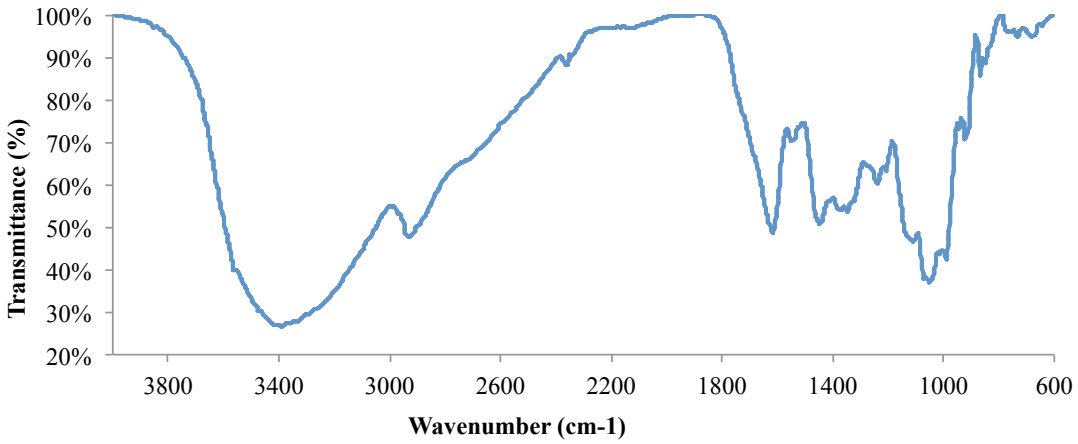


Figure 17. Fourier transform infrared spectrum (FT-IR) of carob kibbles before biosorption prepared with KBr pellet

The broad absorption near 3384 cm^{-1} can be attributed to the vibration stretching of O–H, present in carbohydrates, proteins, and absorbed water [59], [63], [64]. The band in the region of 2935 cm^{-1} suggests the absorption arising from the stretching of C–H of alkane groups [59], [63], [64]. The peak at 1633–1635 cm^{-1} suggests the stretching vibration of C–O double bond of carboxylic groups; the band at 1560 cm^{-1}, in addition to the vibration stretching of C–O, can also be attributed to the N–H stretching present in proteins [63]. The band at 1460 cm^{-1} is due to the skeletal C=C vibrations in aromatic rings [59] and the peak at 1353–1404 cm^{-1} suggests the stretching vibration of inplane O-H bend [64]. The band at 1251 cm^{-1} is associated with the C–O stretching, but in phenols [64], and the band at 1068 cm^{-1} is assigned to O-H bonds and C-OH stretching of phenolic groups [64].

Table 10. Functional groups in dried carob kibbles *(Ceratonia siliqua L)* by using FT-IR spectra

Functional group	Wavenumber (cm^{-1})
Hydroxyl; O-H	3384
Alkyl; C-H	2935
Carbonyl; C=O	1633
Amine; N-H	1560
C=C	1460
C-O phenols	1251
C-O phenols and C-OH	1068

The results of the present FT-IR are similar with the results suggested by M. Farhan et al [57] and by M. Farnane et al [59] who presented both the FT-IR spectra of raw carob shells and identified comparable stretching regions. In addition, the functional groups observed by the spectra are in accordance to the chemical structures of the polyphenols tentatively identified in carob kibbles [54] and to the chemical composition of carob: protein, carbohydrates, and tannins determined by R. Avallone et al [55].

The identification of functional groups in the biomass is important, because the biosorption may be the result of electrostatic interactions and formation of complexes between the sorbate ions and functional groups present in the biosorbent. From the FT-IR spectrum analysis of the carob kibbles powder, it is suggested that this material has functional groups such as hydroxyl, amine, carbonyl and carboxyl groups, which may enable the adsorption of boron. Table 10 summarizes the functional groups in dried carob kibbles found in the present study.

4.1.2 Scanning Electro Microscopy coupled to Energy-Duspersive X-Ray System

Energy-dispersive X-ray spectrum (EDS) enabled the identification of some elements present in the surface of the biomass.

For the natural biomass of carob kibbles figure 18 showed that Ca, C, and O constituted some of the major elements of the carob.

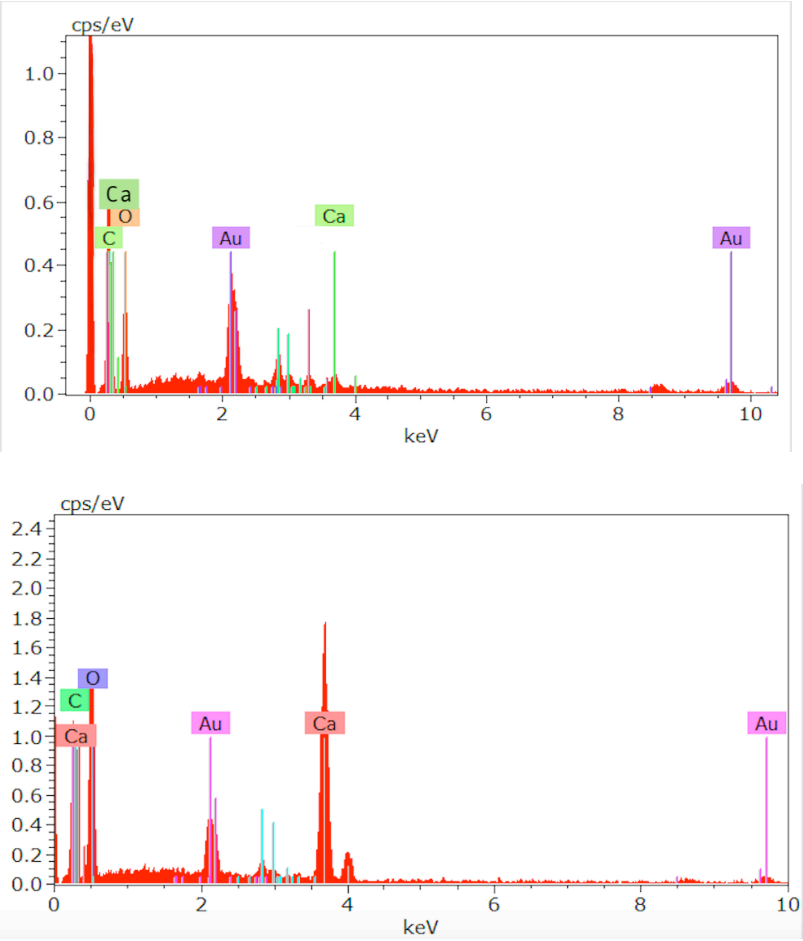


Figure 18. EDX spectrum of natural carob kibbles (top) and carob kibbles enriched with boron (bottom), mineral trace analysis.

4.1.3 Point of zero charge (PZC)

The point of zero charge was determined to know the charge of the carob surface as a function of pH and for a better understanding of the mechanism of the boron biosorption process. After the experiments they were obtained the initial pH of the solution (pHi) and the final pH of the solution (pHf) and the difference between them ($\Delta pH = pH_i - pH_f$) was calculated (Table 9). The point of intersection of the resulting curve with abscissa at which pH = 0 gave the pH_{pzc} (Figure 19).

According to the results, the pH_{pzc} for raw carob kibbles is equal to 5.15, therefore, the biomass surface under study is acidic at pH lower than 5.15 and basic at pH higher than 5.15.

Table 11. Initial and final pH of the solution and ΔpH ($pH_{initial} - pH_{final}$) to determine the point of cero charge (pH_{pzc}) of Carob kibbles (0.1g)

#	pHi	pHf	ΔpH
1	1.80	1.81	-0.01
2	2.40	2.43	0.03
3	3.20	3.75	-0.55
4	4.20	4.81	0.061
5	5.40	5.22	0.18
6	6.20	4.92	1.28
7	9.20	4.38	4.82
8	9.40	3.89	5.51
9	10.00	5.87	4.13
10	11.20	6.08	5.12
11	12.00	8.86	3.14

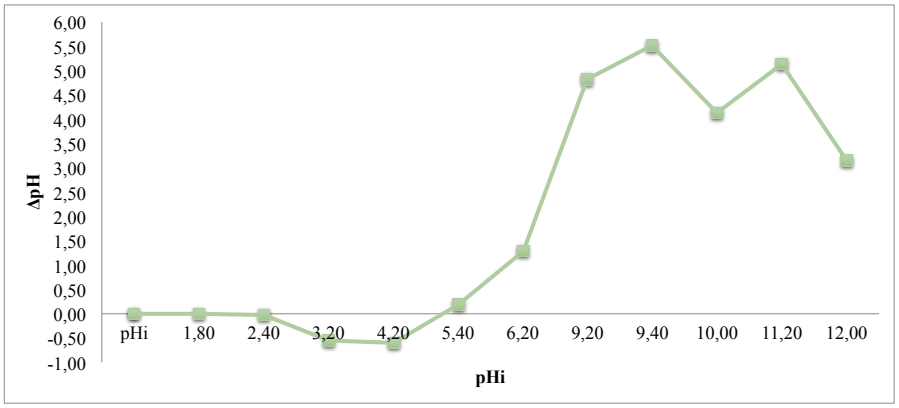


Figure 19. Point of zero charge of carob kibbles before biosorption (0.1g)

The PZC found in this study is in accordance to the reported in the literature [59] where the pH_{pzc} for raw carob shell was equal to 5.4.

4.1.4 Particle size distribution

After the sieve procedure, calculations were made to obtain the percentage of biomass that was retained by each sieve (Table 10). A plot for the retained percentage (%) biomass versus the sieve size (mm) is presented in Figure 20.

Table 12. Percentage of biomass that was retained by each sieve of carob kibbles

Sieve # (mm)	Retained percent (%) n=3
>2.0	0.49±0.17
>1.0-2.0	1.82±0.15
>0.5-1.0	15.0±1.2
>0.2-0.5	22.8±0.39
>0.2-0.25	5.91±0.10
>0.106-0.20	18.6±0.65
>0.025-0.106	32.6±1.2
Base	3.11±0.32

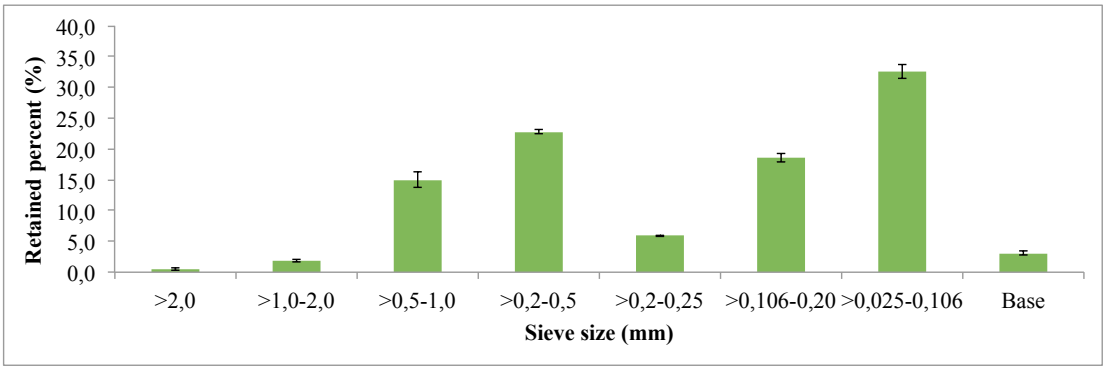


Figure 20. Particle size distribution for carob kibbles expresed as retained percentage (%) biomass (n=3). Bars represent the standard deviation

It can be noticed that in the carob kibbles used as biosorbent most percentage of mass retained (32.6 %) corresponds to particles with size between 0.025 and 0.106 mm, followed by particles with size between 0.25 and 0.5 mm (22.8 %).

4.1.5 Limit of detection

For boron determination by Microwave Plasma Atomic Emission Spectroscopy (MP-AES) the limit of detection calculated from the SDy/x was 1.85 mg B/L.

4.2 Batch biosorption experiments

4.2.1 Kinetic experiments

Kinetics of biosorption of boron by biomass is generally described by pseudo-second-order model [47], [50]. If the pseudo-second-order kinetics is applicable, the plot of t/q_t against t of equation 13 should give a linear relationship, from which q_{eq} and k can be determined from the slope and intercept of the plot. The parameters of pseudo-second-order model are presented in Table 13. The experimental data showed good compliance with the proposed pseudo-second-order model (Figure 21). The regression coefficients for the linear plots were higher than 0.99.

Table 13. The parameters of pseudo-second-order model at different pH (3.0, 5.5, 7.5, 9.5, 11.5)

pH	q_{eq} (mg/g)	k(g/mg.min)	R^2
3.0	20.4	0.0011	0.992
5.5	24.4	0.0015	0.999
7.5	26.8	0.0010	0.997
9.5	30.3	0.0020	0.999
11.5	34.5	0.005	0.996

On the basis of information from table 13, it can be noticed that the highest affinity of the biosorbent to boron (k=0.005) corresponds to pH equal to 11.5, which may suggest that this is the most favorable condition for boron uptake by carob kibbles [32].

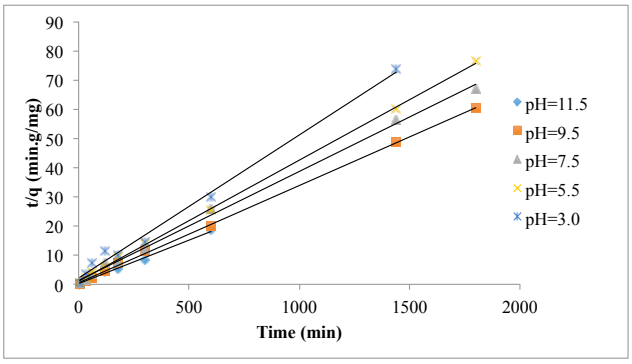


Figure 21. The pseudo-second-order kinetic model plots at different pH (Co=100 mg/L, Cs=50 g/L, 25 ºC)

4.2.2 Effect of contact time

The bioremoval of boron on carob kibbles at Co 100 mg/L, Cs 50g/L and 25 ºC was studied as a function of contact time at a specified pH to determine the time needed to reach the equilibrium for the correspondent pH.

The results of the influence of contact time on adsorption at pH 5.5 are presented in Figure 22.

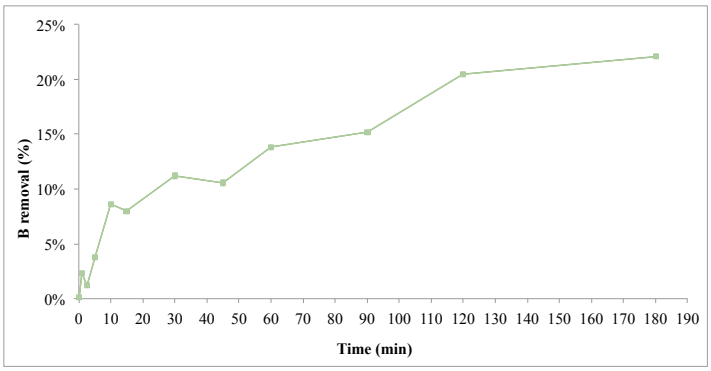


Figure 22. Effect of contact time over boron biosorption by carob kibbles, n=1 (pH=5.5, Co=100 mg/L, Cs=50 g/L, 25 ºC)

As it can be noticed from the plot, apparently, there is a trend to continue the increment of the removal even after the 180 minutes. This means that at this upper time, the boron have not attained the equilibrium. A new set of experiments was done in order to test the boron uptake at longer time.

The effect of contact time at pH 5.5 on boron biosorption for contact time beyond the 180 min is given in Figure 23.

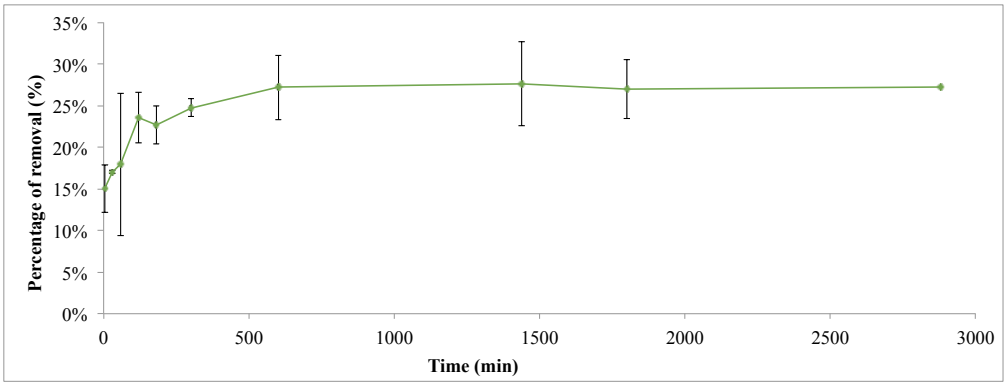


Figure 23. Effect of contact time over boron biosorption by carob kibbles, n=2 (pH=5.5, Co=100 mg B/L, Cs=50 g/L, 25 ºC). For the last point n=1. Bars indicate the standard deviation

The results from Figure 23 showed that for solution pH equal to 5.5 the boron biosorption varies from 15 % (5 min) to 27 % (2880 min). It can also be observed that from minute 600 boron has attained biosorption equilibrium since the percentage of B removal did not increase beyond 27 % after that time. The sorption of boron at Co=100 mg/L on active carbon prepared from olive bagasse [47] was studied as a function of contact time to determine the equilibrium time at pH 5.5 and 25 ºC. The equilibrium time was determined as 48 h. This may suggest that the carob kibbles can bioremove boron faster than active carbon prepared from olive bagasse for the same experimental conditions. However, in the cited reference the amount of biomass was used was different (20 g/L) and shaken at 130 rpm.

For pH 3.0, the biosorption was also tested as a function of contact time (Figure 24).

Results showed that for a solution pH equal to 3.0 the boron biosorption varies from 10 % (5 min) to 21 % (1800 min) with a maximum removal about minute 300, where the biomass has not increased the percentage of boron removal beyond 22 %. Also, it seems that the boron binding accelerates during the first minutes then, it becomes slower for the remaining time of the uptake process.

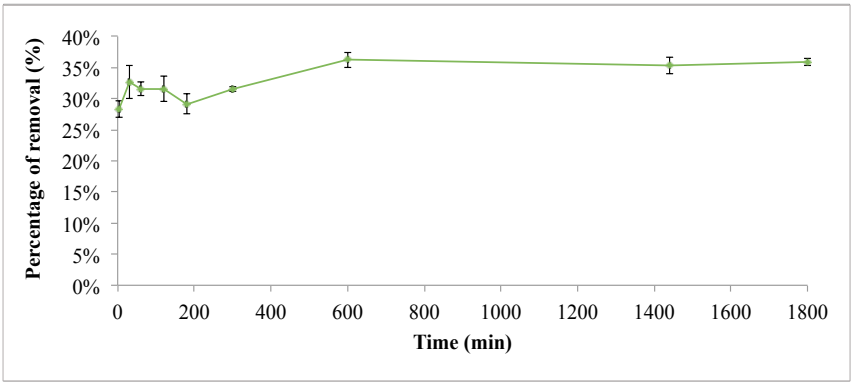


Figure 24. Effect of contact time over boron biosorption by carob kibbles, n=2 (pH=3.0, Co=100 mg B/L, Cs=50 g/L, 25 ºC). For the last point n=1. Bars indicate the standard deviation

For the pH 7.5 the biosorption was also tested as a function of contact time (Figure 25).

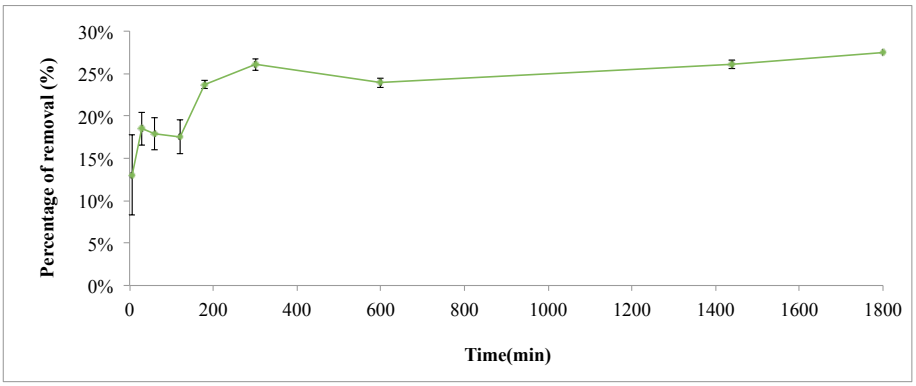


Figure 25. Effect of contact time over boron biosorption by carob kibbles, n=2 (pH=7.5, Co=100 mg B/L, Cs=50 g/L, 25 ºC). For the last point n=1. Bars indicate the standard deviation

From figure 25 it can be noticed that at pH 7.5 during the first 300 minutes the boron uptake takes place in a fast way, then, the biosorption occurs slower for the remaining time. At these conditions, the boron biosorption varies from 13 % (5 min) to 27 % (1800 min).

The biosorption of boron on carob kibbles at pH 9.5 is presented in Figure 26.

For pH of solution 9.5, the biosorption of boron onto carob kibbles develops a rise in the percentage of removal from 28 % at 5 min of contact time to 36 % for the contact time of 1800 minutes.

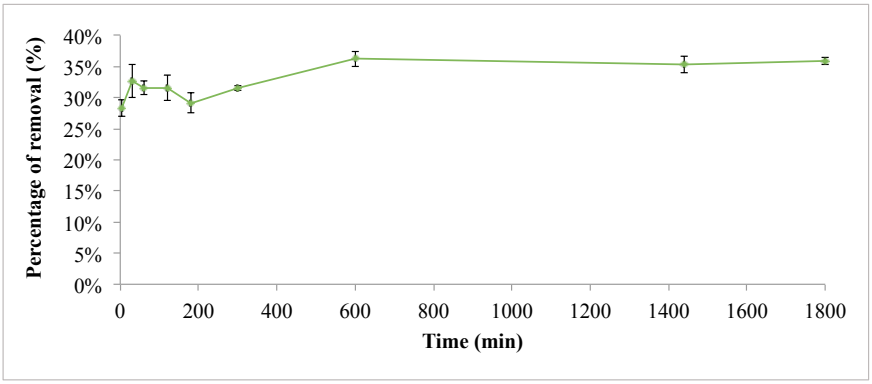


Figure 26. Effect of contact time over boron biosorption by carob kibbles, n=2 (pH=9.5, Co=100 mg B/L, Cs 50 g/L, 25 ºC). For the last point n=1. Bars indicate the standard deviation

The biosorption of boron on carob kibbles at pH 11.5 is presented in Figure 27.

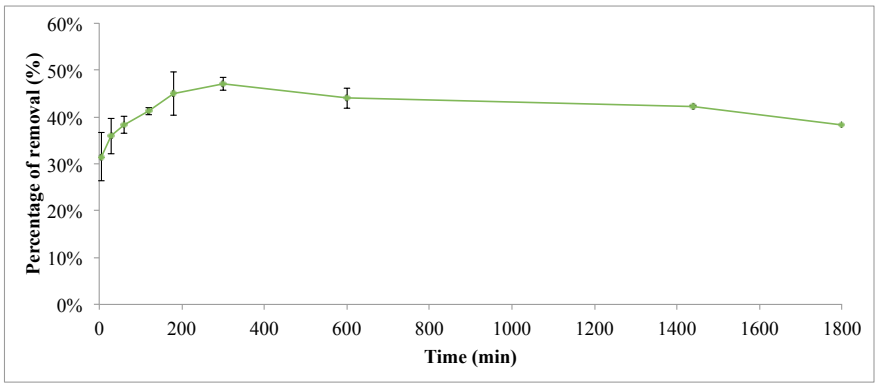


Figure 27. . Effect of contact time over boron biosorption by carob kibbles, n=2 (pH=11,5, Co=100 mg B/L, Cs=50 g/L, 25 ºC). For the last point n=1. Bars indicate the standard deviation

For pH of solution 11.5 the biosorption of boron onto carob kibbles develops a rise in the percentage of removal from 31 % to 38 % during the contact time (5 minutes to 1800 minutes) with a maximum uptake of 47 % of boron removal at 300 minutes.

Comparing with the results for the other pHs of the solutions, 11.5 seems to be a condition that accelerates the boron binding, since during the first five minutes the boron uptake showed a high percentage of removal (31 %).

Studies on the effects of the pH 3.0, 5.5, 7.5, 9.5, 11.5, and contact time on boron biosorption onto carob kibbles biomass are presented in Figure 28

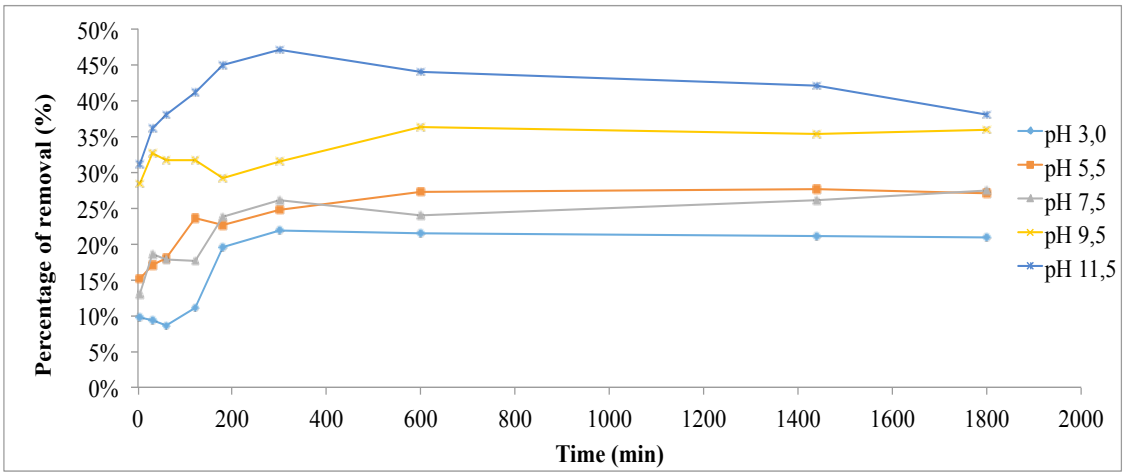


Figure 28. Effect of contact time over boron biosorption by carob kibbles, n=2 (pH=3.0, 5.5, 7.5, 9.5, 11.5, Co=100 mg B/L, Cs=50 g/L, 25 ºC)

From the Figure 28 it can be noticed that in general, the sorption of boron was fast. The equilibrium time were attained in 300 min for pH 3.0 (22 % of boron removal), 600 min for pH 5.5 (boron removal of 27 %), 1800 min for pH 7.5 (27 % of boron removal), 600 min for pH 9.5 (36 % of boron removal) and 300 min for pH 11.5 (47 % of boron removal).

This boost of the boron binding on biomass can be because of the availability of sufficient vacant adsorbing sites in the presence of 100 mg/L boron concentration.

Further increase in contact time beyond 300 min for pH 11.5 did not increase the maximum adsorption probably due to competition for the available adsorption sites in the biomass between borate ions ($B(OH)_4^-$) and the abundant hydroxyl ions (OH^-) in the alkaline conditions.

The sorption equilibrium time was determined as 5 h (300 min) for pH of solution of 11.5, initial boron concentration of 100 mg/L, biomass dosage 50 g/L at 25 °C, since at this contact time the maximum percentage of boron removal was observed even for all the range of pH conditions tested. This results are in accordance to what was found for the kinetics calculations, where the time to obtain the equilibrium corresponds to 300 min (pH of solution equal to 11.5).

According to the literature, boron binding by biosorbents can take from 2.5 hours (for

marine seaweed: *Caulerpa racemosa* var. *cylindracea*) [50] to 48 hours (using activated carbon prepared from olive bagasse, Figure 29) [47] until biosorption equilibrium is established. This implies that depending on the type of biomass, the boron uptake can be a fast or slower process due to the chemical composition of the surface of biomass.

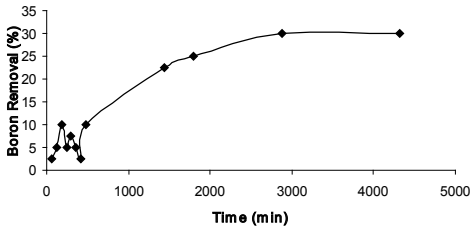


Figure 29. . Effect of contact time on boron removal onto activated carbon (initial pH of solution: 5.5) [47]

In this sense, the optimum contact time established for boron bioremoval on carob kibbles is within the range found in literature.

4.2.3 Effect of pH

The pH of the aqueous solution affects the boron speciation and also affects the surface chemistry of the biosorbent [1], [2], [3], [34]. As outlined in the introduction, boron species are present as boric acid or as borate anions depending on the pH of the solution [1], [2], [3]. Figure 30 represents the effect of pH on boron biosoprtion on carob kibbles at the equilibrium contact time recorded for each pH of solution.

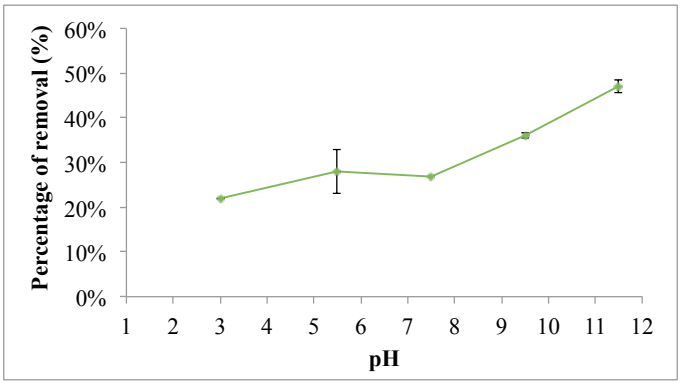


Figure 30. Effect of pH on boron biosorption on carob kibbles n=2 (equilibrium contact time: 300 min for pH=3.0; 1440 min for pH=5.5; 1800 min for pH=7.5; 600 min for pH=9.5; 300 min for pH=11.5; Co=100 mg B/L, Cs=50 g/L, 25 ºC). For pH 7.5 n=1. Bars indicate the standard deviation

During the batch experiments it was observed that at acidic conditions (pH<7), where the major species in solution are boric acid in molecular form, the bioremoval is low (<27 %). For instance, at pH of solution 3.0 it was noticed the lowest percentage of boron uptake (10 %) and at these conditions the maximum boron removal (21 %) was also the minimum value from all the maximum sorbate uptakes observed in all the pH of solutions tested. It can be seen in the results of this study and also in literature that indeed, the acidic conditions supress the binding of boron regardless the presence of biosorbent [47], [48], [49], [50], [51].

From the findings obtained in other studies Figure 31 represents an example of the effect of pH on boron removal onto activated carbon prepared from olive bagasse [47]. The results show that the optimum pH of the solution was 5.5 with approximately 30 % of boron removal which is very close to the maximum uptake found in the present study (28 %) for the same pH of solution. According to the authors, the adsorption is suppressed at low pH because the H^+ ion is a product of the boric acid adsorption .

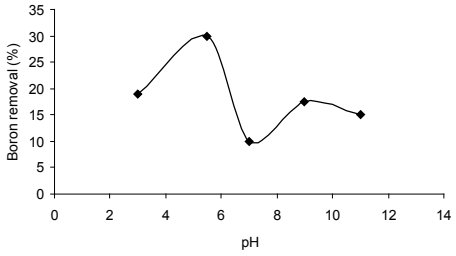


Figure 31. Effect of pH on boron removal onto activated carbon prepared from olive bagasse

The Figures 28 and 30 indicate that the sorption is favored as the pH of the solution increases up to pH=11.5. Taking into account the chemistry of boron in aqueous environment, this can be interpreted as for the carob kibbles the borate ions are better retained than the boric acid. Eventualy, this can be related to the surface charge of carob kibbles in a basic aqueous environment (PZC=5.15). Remarkable, as the biomass surface is negatively charged the borate ions are binding on. This change in the B sorption by pH can be related to the pH-dependence of protonation and deprotonaion of reactive surface functional groups of carob kibbles. The increased pH causes to increase in: (1) negative surface charges on sorbents mainly attributed to the hydroxyl groups and (2) dissociation of H_3BO_3 to $B(OH)_4^-(aq)$ in solution which perhaps has more

affinity to replace with surface OH^- groups in carob kibbles through ligand exchange process [51].

It is observed that the pH of the solution strongly influences the boron biosorption efficiency on carob kibbles and also on several types of biomass, for instance, cotton cellulose or rice husk. For several sources of biomass an optimum pH in the aqueous solution can range between 5 and 9 (Table 14) [47], [48], [49], [50], [51]. From all the pHs of solutions tested in literature, it was interestingly found an optimal pH of 5 for a biomass prepared from rice husk (removal reported as sorption equilibrium capacity of 4.23 mg/g) and the highest optimal pH of solution reported was 7.5 for Marine seaweed: Caulerpa racemosa var. Cylindracea used as biosorbent (63 % of boron removal at equilibrium).

Table 14. Optimal pH values found for the biosorption of boron on biomass

Biomass source	Optimal pH	Reference
Olive bagasse	5.5	47
Cooton cellulose	7	48
Rice husk	5	49
Marine seaweed: Caulerpa racemosa var. Cylindracea	7.5	50
Natural minerals and Organic waste material	9 waste calcite/ 7 rice	51

In this study a pH of 11.5 (300 minutes) was found to be the optimal removal condition because at this value the higher percentage of boron removal was observed (47 %).

Therefore, 300 min and pH 11.5 were used in the subsequent experiments.

This implies that the optimum pH of solution for the present study is higher than the others reported in literature [47], [48], [49], [50], [51].

In other literature [26], [64], [65], it is also reported that some reverse osmosis membranes for water desalinization including boron separation, as well as nanofiltration for boron removal from landfill leachate, require a pH of feedwater close 11 units for the boron rejection.

4.2.4 Effect of initial boron concentration

Adsorption of borate ions by carob kibbles as a function of the initial concentration of boron is shown in Figure 32. The percentage of boron removal decreases with the increase of boron concentration in the solution. The percentage of removal decreases from 59 % to 19 % as the concentration of boron in the aqueous solution was increased from 20 to 200 mg/L. In this regard, the best removal was obtained at 20 mg/L.

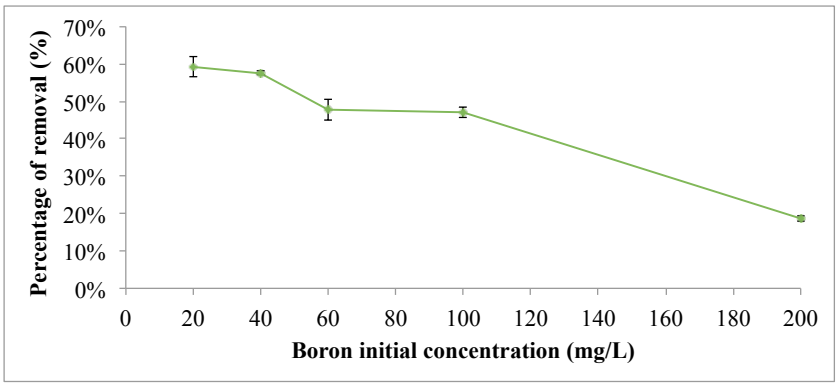


Figure 32. Effect of initial boron concentration on boron removal onto carob kibbles, n=2 (Cs=50 g/L, pH=11.5, 300 min, 25 ºC). Bars indicate the standard deviation

The effect of initial boron concentration on boron adsorption was also investigated for other types of biomass such as olive bagasse, cotton cellulose and rice husk. In general, the results show that the amount of boron sorbed increased with increased boron concentration. In theory an increase in the initial concentration of adsorbate will increase the driving force of the concentration gradient, causing an increase in adsorption capacity which applies for the majority of biomass studied for boron removal.

The findings of the present indicates that adsorption is highly dependent on the boron initial concentration and this behavior can also be attributed to the several aspects: initially, all binding sites on the biomass surface were vacant resulting in high boron biosorption at the beginning [33]. After that, with increasing boron concentration, the biosorption of boron was decreased because of few active sites available on the surface of the carob kibbles biomass [33]. In fact, all of the biosorbents including carob kibbles had a limited number of active sites, which would have become saturated above a certain concentration [44], [66], [67].

4.2.5 Influence of biosorbent dose

Theoretically, as the mass of the biomass increases, there would be more potential surface area to get in contact with the sorbate(s) to be removed. Figure 33 shows that the effect of biomass dosage is important for removing boron. It was observed that the removal of boron increases, as expected, with the increase in the adsorbent dosage from 1 to 20 g in 100 mL solution. The highest boron removal (67 %) was achieved with an adsorbent dosage of 20 g.

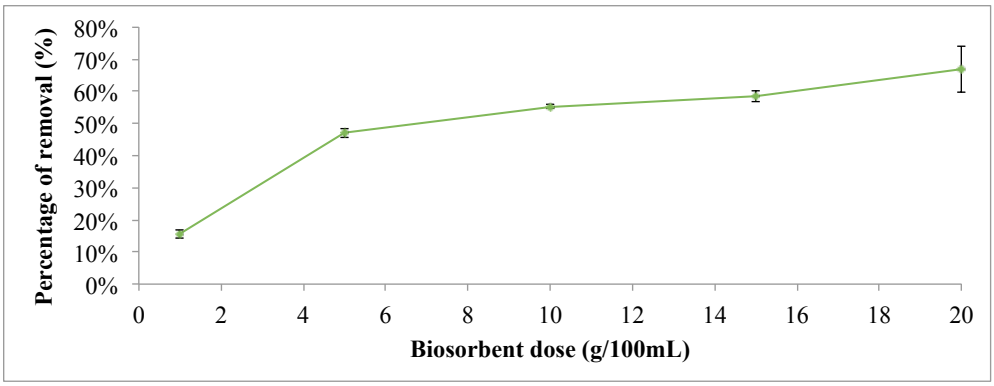


Figure 33. Effect of biosorbent dose on boron removal onto carob kibbles, n=2 (Co=100 mg/L, pH=11.5, 300 min, 25 ºC). Bars indicate the standard deviation

In another study, aiming the sorption of boron by invasive marine seaweed [50], it was demonstrated that boron removal increases until 0.2 g of adsorbent dosage is tested in 25 mL of solution containing 10 mg/L of boron, due to the increase in boron sorption sites. Also, beyond 0.2 g of adsorbent dosage, boron removal remains constant at 50 %. Similarly, for an ion exchange resin, the increase in resin dosage increased the percent of boron removal [68], which is due to the increase in sorbent surface area of the resin. The results also indicate that the removal efficiency increases up to the optimum dosage beyond which the removal efficiency is negligible.

On the other hand, when rice husk was used as biomass for boron removal [49], the adsorption capacity of boron (expressed as qe) decreases with an increase in the adsorbent dosage from 1 to 6 grams for 500 mL of aqueous medium (Co=100 mg/L); in this study the highest removal expressed as boron adsorption capacity (q_e= 38.63 mg/g) was achieved with an adsorbent dosage of 1 g.

4.2.6 Effect of temperature

The rate of boron biosorption does not depend on the temperature at which the process takes place. In this study three temperatures were tested (25 ºC, 45 ºC, 55 ºC). About 47 % boron adsorption was observed at 25 ºC but it slightly decreases for 45 ºC (39 %), although a small increase was observed as the temperature increases to 55 ºC (43 %), (Figure 34). However, on the basis of ANOVA analysis it can be assumed that there are not significant differences between the results (see Annex 2). However, the optimal temperature for adsorption was chosen to be 25 ºC for carob kibbles, which is close to room temperature. This can be important from an economic point of view since no extra energy needs to be spent to achieve better boron removals.

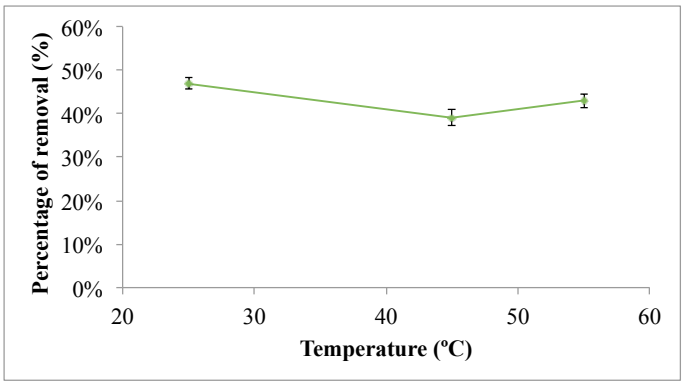


Figure 34. Effect of temperature on boron removal onto carob kibbles, n=2 (Co=100 mg/L, Cs=50 g/L, pH=11.5, 300 min). Bars indicate the standard deviation

These results are slighly different to other previous studies which reported that during the adsorption of boron from aqueous solutions using activated carbon prepared from olive bagasse [47], the sorption of boron decreased as temperature increased, indicating that the sorption process was favored at lower temperatures. These sorption experiments were run at 25, 45 and 55 ºC as working temperatures. Other biosorption experiments aiming to evaluate the boron uptake onto biomasses such as cotton cellulose and rice husk were made at room temperature without assessing the effect of this parameter [48], [49]. In addition, the sorption of boron at 600 mg/L concentration on Dowex 2x8 ion exchange resin was studied as a function of temperature [68]. The sorption of boron decreased as the temperature increased and it was explained that this is an indication that the sorption process was exothermic. The percent of B removal was determined as 55, 40 and 33.3 % at 25, 35 and 45 ºC, respectively.

The effect of temperature on boron sorption was investigated contacting the sorbate with marine seaweed: *Caulerpa racemosa* var. *cylindracea* as biomass. In the experiments, the working temperatures were also 25, 35, 45 ºC. From these results, the amount of adsorbed boron increased with the rise of temperature due to increase in the possibility of interaction between boron and biomass [50]. According to the authors, this behavior in response to temperature was due to the increase in active surface centers available for sorption with temperature.

4.2.7 Effect of particle size

Results showed that particle size affects the boron binding. For the bioadsorption of boron, increase in particle size from 0.025 mm to 1.0 mm decreased the adsorption from 55 to 48 % (Figure 35). The higher boron uptake by smaller particles may take place due to greater surface area for bulk adsorption per unit weight of the carob kibbles.

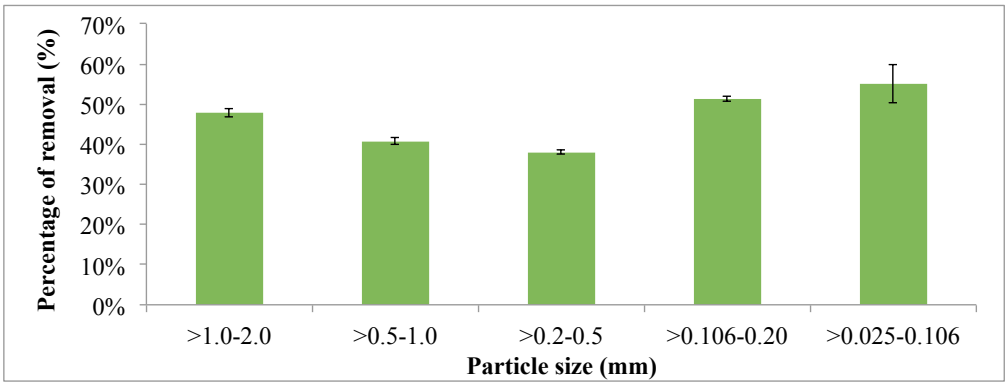


Figure 35. Effect of particle size on boron removal onto carob kibbles, n=3 (Co=100 mg/L, Cs=50 g/L, pH=11.5, 300 min, 25 ºC). Bars indicate the standard deviation

Alternatively, in previous studies for rice husk as biosorbent the percentage of boron removal for three different particle sizes shows that for higher particle size (0.425-1.0 mm), boron uptake was found to be high at 84 %.

Table 15 illustrates a comparison of optimal experimental conditions obtained for boron biosoprtion onto carob kibbles along with results from other studies working with different biomass origin namely olive bagasse, cotton cellulose, rice husk, sea weed and organic waste material. Results for Dowex 2x8 ion exchange resin are also presented.

Table 15. Comparison of optimal experimental conditions obtained for boron biosoprtion

Adsorbent (biomass)	Modification	Optimal experimental conditions						Percentage of removal at equilibrium (%) or boron adsoprtion capacity (mg/g)	qmax from biosorption isotherm (mg/g)	Reference
		pH	Time (h)	Temperature (ºC)	[B] mg/L	Dosage (%)	Other			
Carob kibbles	_	11,5	5	25	100	20	_	67%	0,983	This work
Olive bagasse	Pyrolisis: activation temperature of 850 °C under nitrogen ow with a heating rate of 10 °C min^{-1}.	5.5	48	25	100	2	_	30%	3.5	47
Cooton cellulose	NaOH 0.1M	7	4	Room	500	0.2	_	11.4 mg/g	41.49 (pH=8)	48
Rice husk	_	5	8	Ambient	300	0.1	Optimal particle size: 0.425-1 mm	4.23 mg/g	No isotherm	49
Marine seaweed: Caulerpa racemosa var. Cylindracea	_	7.5	2.5	45	8	0.8	0.1 MNaCl optimal ionic strenght	63%	No isotherm	50
Natural minerals Organic waste material	0.1 M $FeCL_3$(mineral sorbents)-2 L =0.1 M NaOH/100 g (organic residue)	9 (waste calcite)/7 (rice)	24(mineral sorbents)/48(organic sorbents)	_	120	5(mineral sorbents)/0,2(organic sorbents)	0.001 $MCaCL_2$ as background electrolyte for isotherm	0.24 mg/g (waste calcite)-2.9 mg/g(wheat residues)	9.26 (rice residue)	51
Dowex 2x8 ion exchange resin	NaOH 2 M/48 h	9	8	25	600	2	_	55%	3.166	68

4.3 FT-IR analysis of the biosorbent loaded with boron

Once the biosorption experiments were done, a dry sample of carob kibbles loaded with boron was prepared for the FTIR analysis. The spectrum obtained along with the spectrum of the sample without boron is presented in Figure 36. FTIR analysis was conducted with boron-loaded carob kibbles in order to identify groups involved in the biosorption process.

Differences between the two spectra could be seen in the absorbance wave number and intensities.

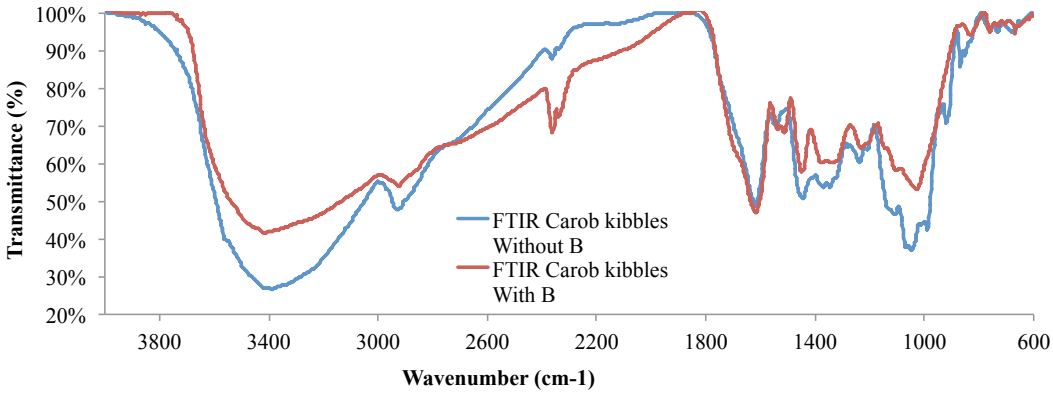


Figure 36. Fourier transform infrared spectrum (FTIR) of carob kibbles before and after boron biosorption

In the region at 3384 cm^{-1} (carob kibbles without B) it can be noticed a change to 3438 cm^{-1} (carob kibbles loaded with B) and also a change in the intensity of the peak is observed which might suggest participation of O-H bending vibrations. This is similar to what was observed in another study on the sorption of boron from aqueous solution onto *Caulerpa racemosa* var. *cylindracea* (CRC) as biomass, where the FTIR spectra of natural and boron loaded biomass showed that OH stretching vibration at 3409 cm^{-1} reduces to 3403 cm^{-1} as a result of boron biosorption.

It can be observed that the peack at 1633 cm^{-1} (carob kibbles without B) which is due to C=O groups, remains the same. Significative changes in the wave number and intensity are observed at 1560 cm^{-1} (carob kibbles without B), 1544 cm^{-1} (carob kibbles loaded with B), 1068 cm^{-1} (carob kibbles without B) and 1053 cm^{-1} (carob kibbles loaded with B), that might suggest participation of N–H and C-O bending vibrations on B adsorption.

Changes in the intensity and minor changes in the wave number [1460 cm^{-1} (carob kibbles without B) 1458 cm^{-1} (carob kibbles with B), 1353 cm^{-1} (carob kibbles without B) 1348 cm^{-1} (carob kibbles with B) and 1251 cm^{-1} (carob kibbles without B) 1245 cm^{-1} (carob kibbles witht B)] might also suggest participation of C=C, O-H and C-O groups on B adsorption, since they are assigned to bending vibrations of those groups.

4.4 Biosorption Isotherms

4.4.1 Langmuir Isotherm

A plot from 1/qeq *vs* 1/Ceq was made and the linearization of Langmuir equation (Figure 37) was obtained.

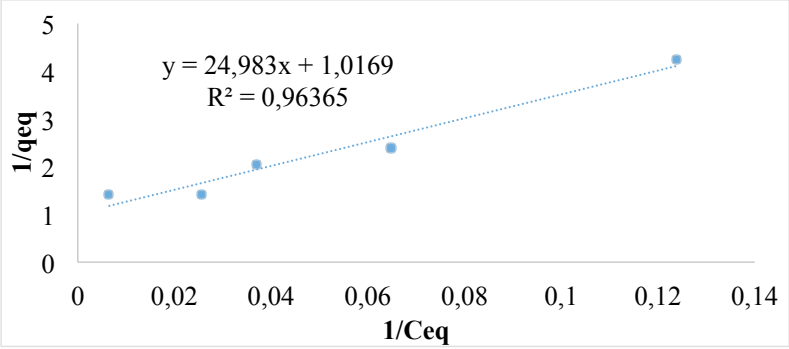


Figure 37. Linearization of Langmuir equation at different boron concentration, 20, 40, 50, 100, 200 mg/L, n=3 (Cs=50 g/L, pH=11.5, 300 min, 25 ºC)

Equilibrium data by the Langmuir isotherm (R^2= 0.964) for 5g of biomas was found to be well fitted in describing adsorption isotherms of carob kibbles as a biosorbent for boron removal.

From linearization the suggested values for K and qmax were 0.0407 L/mg and 0.9833 mg/g, respectively. From these values, qmod was calculated and the Langmuir adsorption isotherm was then plotted (Figure 38).

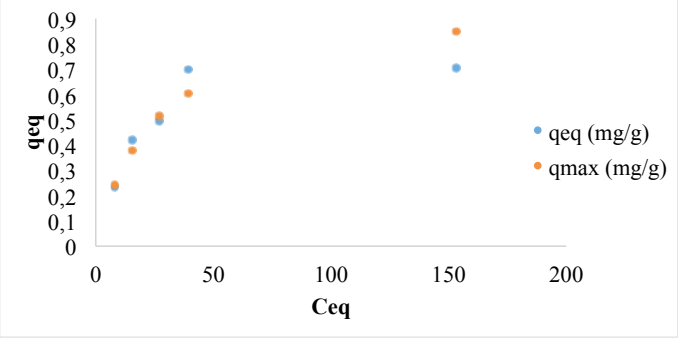


Figure 38. Langmuir isotherm at different boron concentration 20, 40, 50, 100, 200 mg/L, n=3 (Cs=50 g/L, pH=11.5, 300 min, 25 ºC)

It can be seen in the plot (Figure 38) that even the model fits well with the experimental results, it seems that sorption equilibrium is not yet reached at this conditions. This may

suggests that it can be otained a higher qmax and that another model should be tested for the data obtained.

In a study aiming to investigate B removal from aqueous solutions using natural organic waste [51], the qmax obtained was 9.26 (rice residue) and its correspondent K was 0.04 for pH 7 and in the presence of 0.1 M $CaCl_2$. On the other hand, in a study using carbon activated prepared from olive bagasse [47], the equilibrium isotherm for boron binding was described to fit the Freundlich model instead of Lagnmuir isotherm.

The differences in biomass surface chemistry along with different experimental conditions employed during this study and others found in the literature, are the possible causes for the differences of qmax among different biomass for boron removal by bioadsorption including carob kibbles.

4.4.2 Freundlich Isotherm

A plot from ln qeq *vs* ln Ceq was made and the linearization of Freundlcih equation (Figure 39) was obtained.

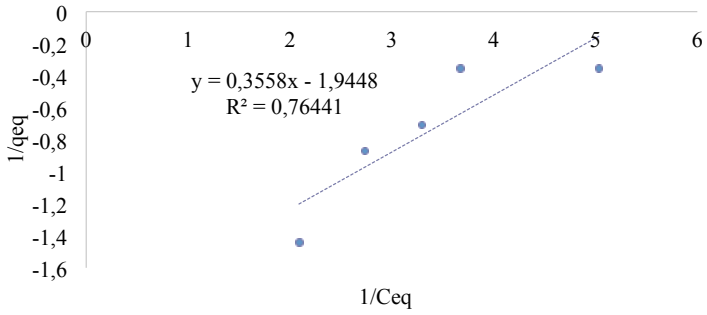


Figure 39. Linearization of Freundlcih equation at different boron concentration, 20, 40, 50, 100, 200 mg/L, n=3 (Cs=50 g/L, pH=11.5, 300 min, 25 ºC)

Equilibrium data by the Freundlich isotherm (R^2= 0.7644) for 5g of biomas was found not to be well fitted in describing adsorption isotherms of carob kibbles as a biosorbent for boron removal.

On the basis of the previously mentioned results for Lagnmuir and Freundlich sorption isotherms, it can be suggested that the model for boron removal by carob kibbles may be another type.

4.5 Batch experiments for the sorption of boron using real samples

Two types of samples of wastewater from ceramic industry were processed with carob kibbles in batch experiments in order to verify the bioremoval of boron on real samples, namely effluent before water treatment and effluent after water treatment. The characterization of the samples was done in the Laboratory of Environmental Technologies of the Center of Marine Sciences in the University of Algarve and are presented in table 16.

Table 16. Characterization of real wastewater solutions

Parameter	Effluent before water treatment	Effluent after water treatment
Boron (mg/L)	36	19
Iron (mg/L)	<1.141	<1.141
Aluminum (mg/L)	1.6	2.9
Copper (mg/L)	<0.531	<0.531
Zinc (mg/L)	<0.143	<0.143
Sulfate (mg/L)	24	10.5
pH	10.45	7.4

The results of boron removal by the real wastewater solutions are given in table 17.

Table 17. Results of boron removal on real samples from a ceramic wastewater n=3 (Cs=200 g/L, pH 11.5, 300 min, 25 ºC)

Sample	Percentage of removal (%) n=3	Recovery (%)
Effluent before water treatment	64.6±0.5	-
Effluent after water treatment	70.2±0.9	-
Fortified blanck (100mL)	-	98

The results in table 17 may suggest that for the given conditions, carob kibbles can remove important amounts of boron from real wastewater solutions. Hence, the complexity of this wastewater does not affect the adsoption of boron. Also, the values of percentage of removal obtained were higher than those achieved with the simulated solutions of boric acid.

4.6 Batch experiments using two different lots of carob kibbles

Two lots of carob kibbles obtained from the same agro food waste but at different dates of the year, were compared on the basis of their perfomance to remove boron from

aqueous solutions. The batch experiments were done using the same following conditions: pH=11.5, Co=100 mg B/L, Cs=50 g/L, 25 ºC. The experiments were done in sixplicate for each lote for a total of 12 samples.

Results of the average and standard deviation for the percentage of boron removal are shown in Table 18. The results of the F-test and the results of the t-Test: Paired Two Sample for Means, two tailed distribution are presented in Table 19 and 20, respectively.

Table 18. Results of the percentage of boron removal using two different lots of samples of carob kibbles (pH=11.5, Co=100 mg B/L, Cs=50 g/L, 25 ºC)

Sample lot	Sampling date	Average (n=6) of the percentage of removal (%)
1	February 2017	43.0±4.0
2	July 2017	41.0±3.0

Table 19. The results of the F-Test

F-test for the two	
F-crit	5.0503
F-cal	1.5204
Fcrit<Fcal	

Table 20. The results of the t-Test: Paired Two Sample for Means, two tailed distribution

t-test for comparing	
t_{crit}	2.228
s	2.457
t	0.4154
$t<t_{crit}$	

Since the calculated value of t is much smaller than the critical value of t (P = 0.05), according to the t-Test: Paired Two Sample for Means, two tailed distribution, there is no significant difference between the results of both samples. Thus, according to this experiment we can assume that similar results were obtained even when different samples of carob kibbles were used.

5. Conclusions

In this study, carob kibbles were used for the first time aiming boron removal from aqueous solutions. The effects of contact time, initial pH of solution, initial boron concentration, biosorbent dose, particle size of biomass and temperature of solution for boron removal from aqueous solutions were evaluated by using non modified carob kibbles (*Ceratonia siliqua L.*) as biosorbent obtained from agrowaste from food industry.

The sorption of boron onto carob kibbles was found to be highly pH dependent. The uptake of boron expressed as percentage of removal was found to decrease with the increase in initial boron concentration and also it is not affected with the increase in the temperature.

The initial boron concentration affects the boron uptake by carob kibbles, less boron is removed from the solution as the initial boron concentration is increased, whereas, an increase in biosorbent dose the removal of boron increases.

Particle size affects boron binding by carob kibbles. The higher boron uptake takes place by smaller particle size.

The biomass of carob kibbles demonstrated the best biosorption properties expressed as percentage of removal (%Rem) in the following conditions: pH 11.5, contact time 300 min, Co=20 mg/L, Cs=200 g/L, temperature 25 ºC, particle size 0,025 mm. However, the transferring the process from the laboratory into industrial scale requires the consideration of economic aspects.

A novel biosorbent with good availability, carob kibbles has been prepared and examined for its potential in removing boron from laboratory aqueous solutions and also from real wastewater samples. Carob kibbles have been found to be a potential biosorbent for the uptake of boron when compared to other types of biomass, already tested.

6. Future persepectives

It is suggested that future research includes kinetic studies for other process parameters including initial boron concentration and biosorbent dose in order to contrast the results with the best process conditions obtained.

Since a model for biosorption isotherm still remains a challenge, it is suggested to check other mathematical models including Brunauer-Emmett-Teller (BET) or Dubinin-Radushkevich (DR).

It is suggested that future research focuses on the boron removal on carob kibbles by using a continuous or semicontinuous approach, since this type of experiments can give additional information on the behavior of biosorption performance of the carob kibbles. In this order of ideas, it would be attractive to test the reusing of the biomass more than once.

On the basis of the point of zero charge of the biomass, it can be investigated the removality of other toxic metals that may include lead, mercury or the metalloid arsenic.

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8. Annexes

Annex 1. t distribution critical values

t Table

cum. prob	$t_{.50}$	$t_{.75}$	$t_{.80}$	$t_{.85}$	$t_{.90}$	$t_{.95}$	$t_{.975}$	$t_{.99}$	$t_{.995}$	$t_{.999}$	$t_{.9995}$
one-tail	**0.50**	**0.25**	**0.20**	**0.15**	**0.10**	**0.05**	**0.025**	**0.01**	**0.005**	**0.001**	**0.0005**
two-tails	**1.00**	**0.50**	**0.40**	**0.30**	**0.20**	**0.10**	**0.05**	**0.02**	**0.01**	**0.002**	**0.001**
df											
1	0.000	1.000	1.376	1.963	3.078	6.314	12.71	31.82	63.66	318.31	636.62
2	0.000	0.816	1.061	1.386	1.886	2.920	4.303	6.965	9.925	22.327	31.599
3	0.000	0.765	0.978	1.250	1.638	2.353	3.182	4.541	5.841	10.215	12.924
4	0.000	0.741	0.941	1.190	1.533	2.132	2.776	3.747	4.604	7.173	8.610
5	0.000	0.727	0.920	1.156	1.476	2.015	2.571	3.365	4.032	5.893	6.869
6	0.000	0.718	0.906	1.134	1.440	1.943	2.447	3.143	3.707	5.208	5.959
7	0.000	0.711	0.896	1.119	1.415	1.895	2.365	2.998	3.499	4.785	5.408
8	0.000	0.706	0.889	1.108	1.397	1.860	2.306	2.896	3.355	4.501	5.041
9	0.000	0.703	0.883	1.100	1.383	1.833	2.262	2.821	3.250	4.297	4.781
10	0.000	0.700	0.879	1.093	1.372	1.812	2.228	2.764	3.169	4.144	4.587
11	0.000	0.697	0.876	1.088	1.363	1.796	2.201	2.718	3.106	4.025	4.437
12	0.000	0.695	0.873	1.083	1.356	1.782	2.179	2.681	3.055	3.930	4.318
13	0.000	0.694	0.870	1.079	1.350	1.771	2.160	2.650	3.012	3.852	4.221
14	0.000	0.692	0.868	1.076	1.345	1.761	2.145	2.624	2.977	3.787	4.140
15	0.000	0.691	0.866	1.074	1.341	1.753	2.131	2.602	2.947	3.733	4.073
16	0.000	0.690	0.865	1.071	1.337	1.746	2.120	2.583	2.921	3.686	4.015
17	0.000	0.689	0.863	1.069	1.333	1.740	2.110	2.567	2.898	3.646	3.965
18	0.000	0.688	0.862	1.067	1.330	1.734	2.101	2.552	2.878	3.610	3.922
19	0.000	0.688	0.861	1.066	1.328	1.729	2.093	2.539	2.861	3.579	3.883
20	0.000	0.687	0.860	1.064	1.325	1.725	2.086	2.528	2.845	3.552	3.850
21	0.000	0.686	0.859	1.063	1.323	1.721	2.080	2.518	2.831	3.527	3.819
22	0.000	0.686	0.858	1.061	1.321	1.717	2.074	2.508	2.819	3.505	3.792
23	0.000	0.685	0.858	1.060	1.319	1.714	2.069	2.500	2.807	3.485	3.768
24	0.000	0.685	0.857	1.059	1.318	1.711	2.064	2.492	2.797	3.467	3.745
25	0.000	0.684	0.856	1.058	1.316	1.708	2.060	2.485	2.787	3.450	3.725
26	0.000	0.684	0.856	1.058	1.315	1.706	2.056	2.479	2.779	3.435	3.707
27	0.000	0.684	0.855	1.057	1.314	1.703	2.052	2.473	2.771	3.421	3.690
28	0.000	0.683	0.855	1.056	1.313	1.701	2.048	2.467	2.763	3.408	3.674
29	0.000	0.683	0.854	1.055	1.311	1.699	2.045	2.462	2.756	3.396	3.659
30	0.000	0.683	0.854	1.055	1.310	1.697	2.042	2.457	2.750	3.385	3.646
40	0.000	0.681	0.851	1.050	1.303	1.684	2.021	2.423	2.704	3.307	3.551
60	0.000	0.679	0.848	1.045	1.296	1.671	2.000	2.390	2.660	3.232	3.460
80	0.000	0.678	0.846	1.043	1.292	1.664	1.990	2.374	2.639	3.195	3.416
100	0.000	0.677	0.845	1.042	1.290	1.660	1.984	2.364	2.626	3.174	3.390
1000	0.000	0.675	0.842	1.037	1.282	1.646	1.962	2.330	2.581	3.098	3.300
z	0.000	0.674	0.842	1.036	1.282	1.645	1.960	2.326	2.576	3.090	3.291
	0%	50%	60%	70%	80%	90%	95%	98%	99%	99.8%	99.9%
	Confidence Level										

Annex 2. One way ANOVA for checking the effect of temperature over boron removal by carob kibbles

Average of percentage of boron removal	Standard deviation
47% (25ºC)	0.0135158
39% (45ºC)	0.0177682
43% (55ºC)	0.0161553
Mean (means): 43%	
Standard deviation (means): 0.0396321	
Standard deviation (population): 0.0228816	Mean (standard deviation): 0.0158131
(s temperature)^2: 0.0005235	(s random)^2: 0.0002500
Fcal	(s temperature)^2/(s random)^2: 2.0938127
Fcrit	9.55
Fcal<Fcrit	
There is no difference between the two variances	
There is not significant difference between the groups	